Volume XXX August, 1930 Number 2

SOIL SCIENCE

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PUBLISHED MONTHLY
THE WILLIAMS & WILKINS COMPANY
MT. ROYAL AND GUILFORD AVENUES
BALTIMORE, MARYLAND, U. S. A.

Made in United States of America

SOIL SCIENCE

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EXCHANGEABLE CATIONS AND LIME REQUIREMENT IN DIFFERENTLY FERTILIZED SOILS[1]

S. RAVIKOVITCH[2]

New Jersey Agricultural Experiment Station

Received for publication December 21, 1929

The application of different fertilizers to the soil can be considered as an important factor affecting the amounts and kind of cases in the soil exchange complex. The soluble fertilizers introduced into the soil, change the concentration of the soil solution, which is established as a result of equilibrium reached between the dissolved salts of the soil and the exchangeable cations of the exchange complex. As a consequence of the change in the concentration of the soil solution, an exchange reaction between the cations of the salt introduced and the cations of the exchange complex occurs. A part of the cations of the salts is absorbed, and an equivalent amount of the exchangeable cations appears in the solution.

The amount of the bases in the complex can be increased or decreased, depending on the nature of the fertilizers applied to the soil. Hissink (10) points out the effect of the so-called acid and alkaline fertilizers on the exchangeable calcium in soils. The acid fertilizer is found to bring a marked decrease in the content of the exchangeable bases, as compared with the effect of the alkaline fertilizers. Page and Williams (25) emphasize the influence of different fertilizers on the proportion and content of the exchangeable bases in the Rothamsted soils. The results were obtained from fertilized plots which were chalked. By applications of gypsum and of potassium and magnesium salts, the cations of these salts were introduced into the exchange complex. The addition of chalk to the soils increased the amount of exchangeable calcium. Merkle (23) found that fertilizers and organic manure change the quantity and proportion of the exchangeable cations. Ammonium and potassium when applied as fertilizers are retained by the acidoid complex.

The introduction of the different fertilizers to the soil over a period of years is expected to exert a sensible effect on the soil exchange complex; this effect

[1] Part of a thesis presented to the faculty of Rutgers University and the State University of New Jersey in partial fulfillment of the requirements of the degree of master of science. Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

[2] The writer wishes to express sincere appreciation to Dr. Sante Mattson for his kind suggestions and criticism in the performance of this work. Thanks are due also to Prof. A. W. Blair for placing at his disposal the experimental plots and for the kind interest shown throughout this work.

being more marked in soils which are low in soluble salts and have a low base exchange capacity.

Observations on the effect produced by fertilizers on the state of the soil, are mostly related to certain chemical changes, especially those in the soil reaction. Few invesigators have studied the effect of the different fertilizers on the kind and amount of exchangeable bases of soils.

The present work was performed to study the effect produced by different fertilizers in a Sassafras loam soil in 20 years of fertilization on the content of the exchangeable calcium and magnesium in the soil, on the degree of unsaturation, and on the lime requirement and reaction. This work gave an opportunity to study the effect produced by the fertilizers in the presence and absence of lime in soil.

SOILS USED

Samples of soil taken at a depth of 6½ inches from differently fertilized plots of the New Jersey Agricultural Experiment Station were air dried and passed through a 1-mm. sieve for this study. The soil is a Sassafras loam and has a low exchange capacity—4.50 and 5.69 milliequivalents of bases to 100 gm of soil for the unlimed and limed soils of the check plots respectively. The ratio of silica to sesquioxides of the colloidal part of an adjacent virgin Sassafras soil is 1.66. The sandy character of this soil and the low ratio of $\frac{SiO_2}{Al_2O_3 + Fe_2O_3}$ explain the fact of the low base exchange capacity of this soil. The silica sesquioxide ratio of the colioids is found by Mattson (20) to be directly related to their property to adsorb bases. This investigator observed that with the increase of the ratio of the silica to sesquioxides, there is an increase in the capacity of adsorption by the colloid.

The experimental plots were established in 1908 by Lipman and Blair (13) for the study of the availability of the different nitrogenous fertilizers. The plots receiving different treatments are divided into two sections: A and B. Plots A have received no lime for the last 20 years, whereas plots B has received lime at intervals of 5 years (2 tons ground limestone to the acre). The fertilizer treatment is the same for the corresponding plots in each series (13, 14, 15, 16). Eighteen plots were selected for the experiments, nine from the unlimed section A and nine from the corresponding limed section B. The fertilizer treatments of the plots selected were as follows:

Plot No.	*Annual fertilizer treatment for 1/20-acre plot*
1A, 1B	Nothing
2A, 2B	16 pounds muriate of potash
3A, 3B	32 pounds superphosphate
4A, 4B	Minerals only*

* Minerals—32 pounds superphosphate and 16 pounds muriate of potash. (Since 1923, some changes have been made in the application of superphosphate and muriate of potash, which were reduced to one-half of the original amount, that is, 16 and 8 pounds respectively to each plot.)

Plot No.	*Annual fertilizer treatment for 1/20-acre plot*
5A, 5B	Minerals, 1600 pounds cow manure
9A, 9B	Minerals, 16 pounds $NaNO_3$
10A, 10B	Minerals, $Ca(NO_3)_2$ equivalent to 16 pounds $NaNO_3$
11A, 11B	Minerals, $(NH_4)_2SO_4$ equivalent to 16 pounds $NaNO_3$
12A, 12B	Minerals, $CaCN_2$ equivalent to 16 pounds $NaNO_3$

METHODS

In performing this work, difficulties were met with in applying some of the methods; therefore, more detailed study was necessary. This paper includes the results of the study and a comparison of certain methods which were used afterward in the carrying out of this research.

To determine the amount of the exchangeable calcium in the unlimed soils A, Gedroiz's (7) method was used. Twenty-five gram samples were treated with a neutral normal solution of ammonium chloride. Half a liter of this salt solution was found to be sufficient for complete replacement of the exchangeable calcium in the soil.

In the limed soils B, the determination of the exchangeable calcium was more difficult. Hissink's method (9) was applied. This method consists of leaching a 25-gm. sample of soil with a neutral normal solution of sodium chloride. Hissink assumes that the first portion of the filtrate, equal to 1 liter, contains both the exchangeable calcium and the calcium carbonate brought into the solution by the action of the sodium chloride, and that the second liter contains the dissolved calcium carbonate, equal in amount to that of the first liter. The difference between the amounts of calcium in the first and second liters of the filtrate represents the amount of the exchangeable calcium present in the soil.

In the case of the Sassafras B soils, with a low base exchange capacity, 12.5-gm. samples were treated with sodium chloride solution and two half-liter portions of filtrates were collected and the calcium was determined. By subtracting the amount of calcium found in the second portion of the filtrate from that in the first portion, it appeared that the amounts of exchangeable calcium were too high. The sum of the exchangeable calcium and magnesium in most cases surpassed the total base exchange capacity of the soils tested, even when sodium, potassium, and hydrogen originally present in the soil exchange complex were not taken into consideration. The cause of this phenomenon was supposed to be due to the unequal solubility effect produced by the sodium chloride solution on the calcium carbonate of the soil in the first half-liter treatment and in the subsequent second half-liter treatment, the solubility being higher in the former case.

Two other methods, those of Tyurin (26) and Gedroiz (7) were then applied for determining the amount of the dissolved calcium carbonate in the first half liter of the sodium chloride solution. Tyurin recently suggested, for ascertaining the dissolved carbonates, a method which consists in titrating a portion of the filtrate containing both the exchangeable and the dissolved cal-

cium carbonate with a 0.02 *N* solution of hydrochloric acid in the presence of methyl orange. From the amount of hydrochloric acid used for the decomposition of the carbonates present in the solution, the sum of calcium oxide and magnesium oxide is calculated in terms of calcium oxide, because of the predominance of calcium carbonate in the salt solution. Determinations of the dissolved calcium carbonate were made by this method. The absence of magnesium carbonate or the presence of only traces permitted the calculation in equivalents of calcium from the results obtained by titration.

Gedroiz's method for determining the dissolved calcium carbonate consists in estimating the carbonates in the soil before and after treatment with the salt solution. The difference between the carbonates found before and after treatment gives the amount of the carbonates dissolved.

TABLE 1

Amount of calcium carbonate dissolved from the soils by the normal sodium chloride solution, as determined by different methods

PLOT NO.	CaO DISSOLVED FROM 12.5 GM. OF SOIL			$CaCO_3$ PRESENT IN THE SOIL
	Titration method (Tyurin)	Difference in content of $CaCO_3$ before and after treatment with *N* NaCl solution (Gedroiz)	Ca found in the second half liter of NaCl solution (Hissink)	
	mgm.	*mgm.*	*mgm.*	*per cent*
1B	5.2	5.2	2.4	0.28
2B	3.1	3.6	0.7	0.06
3B	4.5	6.0	1.5	0.16
4B	4.3	4.1	1.0	0.09
5B	4.5	4.6	1.4	0.05
9B	3.4	3.5	0.8	0.06
10B	4.5	4.6	1.4	0.13
11B	3.2	3.8	0.8	0.08
12B	4.6	5.0	1.1	0.08

The amounts of calcium carbonate dissolved, as determined by Hissink's, Tyurin's, and Gedroiz's methods, are presented in table 1. A comparison of the results shows that according to Tyurin's method the amounts of the calcium carbonates dissolved are almost similar to those obtained by Gedroiz's method. In the case of Hissink's method, the results are found to be much lower. By introducing a correction for the dissolved calcium carbonate according to the Hissink method, the amount of the exchangeable calcium is thus found to be too large. Hissink's method gives values for the exchangeable calcium which are greater than those of the two other methods by 0.68 to 1.33 milliequivalent to 100 gm. of soil for the different plots. These amounts introduce a fairly considerable error in soils with a low exchange capacity such as Sassafras loam.

Menchikowsky and Ravikovitch (22) used the method of Hissink for the determination of the exchangeable calcium in highly calcareous soils (15.5 to

40.1 per cent $CaCO_3$). The results obtained showed that the second liter of sodium chloride solution contained almost a constant concentration of calcium carbonate dissolved for the different soils examined. For the Sassafras soils B, with a low content of calcium carbonate (table 1), the constancy of concentration of the calcium carbonate dissolved was not observed. This constancy of solubility produced by the sodium chloride solution may be expected in soils with a high content of calcium carbonate.

The exchangeable hydrogen (unsaturation) was determined by Mattson's calcium carbonate paste method (21), which is based on the principle of interaction between an unsaturated soil and calcium carbonate paste through a parchment membrane. As a result of this contact with the paste, the hydrogen of the complex is replaced by the calcium. Mattson (18) observed that when calcium carbonate is mixed with a soil in the presence of water, as a result of the hydrolysis of the calcium carbonate an amount of calcium is adsorbed by the soil and an equivalent amount of calcium is left in the solution as calcium bicarbonate. The reaction of hydrolysis of calcium carbonate is shown in the following scheme:

$$\left.\begin{array}{l} 2\ H_2O \rightleftarrows 2\ H^+ + 2\ OH^- \\ 2\ CaCO_3 \rightleftarrows 2\ CaCO_3 \rightleftarrows 2\ CO_3^{--} + 2\ Ca^{++} \end{array}\right\} \rightleftarrows Ca(OH)_2 \text{ adsorbed}$$

solid — dissolved

$$\downarrow\uparrow$$

$$2\ HCO_3^- = Ca(HCO_3)_2$$

The calcium hydroxide formed as a result of hydrolysis of the calcium carbonate, is adsorbed by the soil until it is in equilibrium.

The 12.5-gm. samples of soils to be tested were placed in conical bags of thin parchment paper, which were then put into similar shaped bags made of filter paper. Then bags containing the soil were placed in a calcium carbonate paste. The soils were then saturated with water and allowed to remain in the paste until equilibrium between the calcium carbonate and the soil was established. The filter paper bag was introduced throughout this work to keep the parchment bag from direct contact with the solid calcium carbonate and thus to prevent it from sticking to the parchment bags. After equilibrium was established, the inner parchment bag containing the soil was then removed for analysis.

A more detailed study of this method established the fact that during the first period of contact of the soil with the paste, the samples tested contained amounts of calcium (as determined by treatment with ammonium chloride and sodium chloride solutions) sometimes higher than the total base exchange capacity. This rise in calcium content is due to the formation in the soil mass of calcium bicarbonate, which appears simultaneously with the calcium hydroxide. As a result of the intensive adsorption of the calcium hydroxide by the soil in the early stages, the calcium bicarbonate accumulates temporarily in the soil solution. The accumulation of the bicarbonates on the inside of

the membrane produces a diffusion gradient toward the outside paste solution which has a lower concentration of this salt and a higher pH, and thus the calcium bicarbonate is gradually removed from the soil.

The preliminary experiments showed that a period of 21 days is necessary for complete saturation of the soils and that a period of 27 days is sufficient for complete removal of the excess calcium bicarbonate from the soil, as equilibrium is already established. By leaching the samples with water before treatment with the salt solutions, the calcium bicarbonate present in the soil mass after the saturation is completed, can be removed. The results obtained for the amount of calcium after 27 days of contact with the paste, and the same

TABLE 2

Amount of exchangeable bases in the unlimed and limed soils

UNLIMED				LIMED						
	CaO				CaO			MgO		
Plot no.	Per cent	Milliequivalents in 100 gm. soil	Ca_1 in per cent of the total base exchange capacity	Plot no.	Per cent	Milliequivalents in 100 gm. soil	Ca_1 in per cent of the total base exchange capacity	Per cent	Milliequivalents in 100 gm. soil	Mg_1 in per cent of the total base exchange capacity
1A	0.057	2.05	45.5	1B	0.141	5.03	88.4	0.007	0.36	6.3
2A	0.039	1.40	30.4	2B	0.112	3.98	72.4	0.008	0.40	7.3
3A	0.074	2.65	50.2	3B	0.136	4.84	80.3	0.006	0.32	5.3
4A	0.063	2.25	46.0	4B	0.144	5.15	80.5	0.005	0.24	3.8
5A	0.075	2.65	48.2	5B	0.190	6.77	80.7	0.020	0.95	11.3
9A	0.075	2.65	51.1	9B	0.126	4.48	78.1	0.009	0.44	7.5
10A	0.074	2.63	52.3	10B	0.136	4.86	82.2	0.009	0.44	7.4
11A	0.011	0.40	8.7	11B	0.109	3.90	68.7	0.008	0.40	7.0
12A	0.075	2.66	59.9	12B	0.143	5.09	78.4	0.016	0.78	12.0

soils after 21 days of contact when washed before treatment with the salt solution, were found to be identical.

After equilibrium was reached, the A soil samples were treated with ammonium chloride solution and the B soils with sodium chloride solution. In the filtrate the calcium, which was determined, represents the sum of the exchangeable and the adsorbed calcium, and in the case of the limed B soils, also the dissolved calcium carbonate. The difference between the amount of calcium before and after contact with the paste is the calcium adsorbed, which, expressed in milliequivalents to 100 gm. of soil, represents the H-ion content in the soil exchange complex.

The total base exchange capacity was determined by treating the soil with a neutral normal solution of barium chloride until the cations present in the complex were replaced by barium ions.

The hydrogen-ion concentration of the soils examined was determined potentiometrically by the quinhydrone electrode.

The determinations of silicon, aluminum, and ferric oxides in the colloidal part of the Sassafras soil were made by fusion with sodium carbonate (2).

All calculations are made on the basis of oven-dry matter.

EXCHANGEABLE BASES IN THE SOILS

Influence of lime on the exchangeable calcium content

The amounts of exchangeable calcium for plots A and exchangeable calcium and magnesium for plots B are given in table 2 and presented graphically in figure 1.

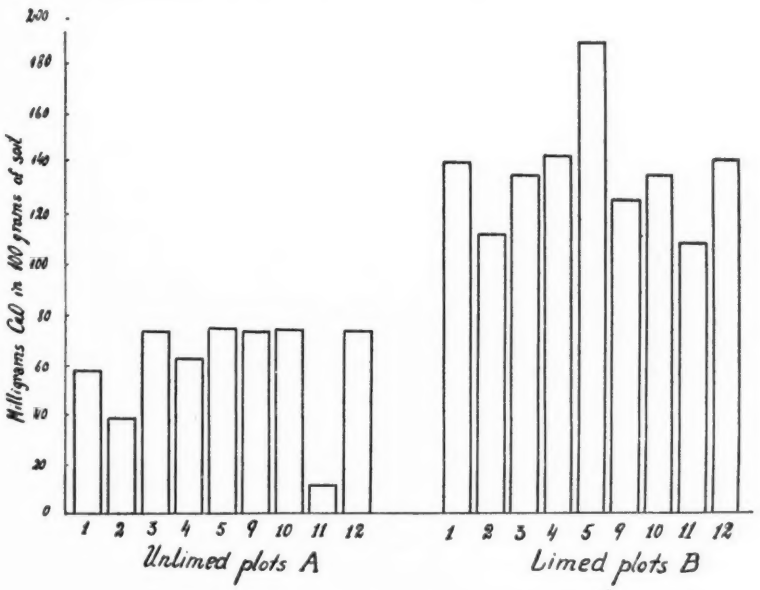

FIG. 1. AMOUNT OF EXCHANGEABLE CALCIUM IN UNLIMED AND LIMED SOILS

From the results obtained, the difference between the amounts of the exchangeable calcium in plots A and B is readily seen. The calcium content of plots B considerably exceeds that of plots A. The increase in the exchangeable calcium content in the B plots is due to the introduction of lime to these soils. Thus this marked difference explains clearly the influence of the liming on the content of exchangeable calcium in the soils. Table 3 shows the effect of liming ($CaCO_3$) upon the amounts of exchangeable calcium in the soils. The increase in the percentage of exchangeable calcium in the B plots, due to the liming, varies from 40.6 per cent to 89.7 per cent.

Influence of fertilizers on exchangeable calcium content

A comparison of the amounts of the exchangeable calcium for the different plots in each of the two series A and B, shows a difference in their content, which is due to the influence of the different fertilizers.

In series A, the exchangeable calcium is lowest in plots 11, 2, and 1. The remainder of the plots, with the exception of 4A, contain equal amounts of exchangeable calcium (2.63 to 2.68 milliequivalents to 100 gm. of soil).

Plot 2A, receiving the muriate of potash, lost a considerable part of its exchangeable calcium. Plot 4A, receiving calcium phosphate, in addition to muriate of potash, contains a higher amount of exchangeable calcium than plot 2A, receiving only muriate of potash. The calcium phosphate, with its constituent calcium sulfate, tends to increase the amount of the exchangeable

TABLE 3

Effect of liming upon the amount of exchangeable calcium in the soils

PLOT NO.	DIFFERENCE IN EXCHANGEABLE CALCIUM BETWEEN THE LIMED AND UNLIMED SOILS		INCREASE OF EXCHANGE CALCIUM IN LIMED PLOTS, DUE TO LIMING
	Per cent CaO	Milliequivalents CaO to 100 gm. soil	
			per cent
1B	0.084	2.98	59.2
2B	0.073	2.58	64.8
3B	0.062	2.19	45.2
4B	0.081	2.90	56.3
5B	0.115	4.09	60.4
9B	0.051	1.82	40.6
10B	0.062	2.23	45.9
11B	0.098	3.50	89.7
12B	0.068	2.43	47.5

calcium in the soil, and, in mixture with the muriate of potash, suppresses the replacing effect of the latter on the exchangeable calcium.

Plot 5A, 9A, 10A, and 12A, receiving in addition to muriate of potash and superphosphate, different nitrogenous fertilizers, such as cow manure, sodium nitrate, calcium nitrate, and calcium cyanamid, respectively, are about equal in their content of exchangeable calcium. These different nitrogenous fertilizers did not induce any comparative changes in the content of exchangeable calcium. The nearly constant amount of the exchangeable calcium in these plots can be related to a balance between the augmenting and suppressing effect of the superphosphate and muriate of potash respectively.

Calcium phosphate and muriate of potash, brought into soils A, create in the solution a definite concentration of these salts. The exchangeable calcium is thus maintained at an equilibrium with the soil solution, and is held at a certain level, which is regulated by the concentration of the calcium and potassium ions of the salts applied. When the exchangeable complex is subjected

to the action of only the potassium ion of the muriate of potash, as in the case of the plot 2A, the effect of this ion is expected to be different from that of the calcium ion of the superphosphate. In this plot the potassium, not having the competition of calcium, exerted its action by removing the calcium from the exchangeable complex. The continual introduction of the muriate of potash into the soil in the course of 20 years, at the rate of 320 or 160 pounds an acre, reduced the amount of the calcium over 30 per cent, as compared with the check plot 1A.

A very considerable change in the content of the exchangeable calcium in the soil was produced by the application of the ammonium sulfate. This salt reduced the amount of the exchangeable calcium to the very low level of 0.4 milligram equivalent to 100 gm. of soil. As a physiological acid salt, the ammonium sulfate brings into the soil two acid radicals, nitrate and sulfate (the first appearing as the result of nitrification). The acids formed, continuously attack the soil exchange complex and remove the combined bases as salts of the nitric and sulfuric acids. The annual introduction of new portions of this salt to the soil, decidedly exhausted the soil of its exchangeable calcium content, which is 85 per cent lower than the plots receiving other forms of nitrogenous fertilizers.

The comparatively lower content of the exchangeable calcium in the check plot 1A can be explained by the fact that this plot did not receive, as did the other plots, calcium phosphate, which leads to a certain increase of the exchangeable calcium.

The exchangeable calcium content of the limed plots of series B varies. In those plots the effect of muriate of potash and of ammonium sulfate in decreasing the content of the exchangeable calcium can also be observed. This influence is less pronounced than in the unlimed plots 2A and 11A on account of the presence of calcium carbonate, which maintains the saturation of the soil.

Plot 5B, receiving cow manure, surmounts the other plots in its content of exchangeable calcium. This high content is related to the high exchange capacity of the soil. The higher capacity is due to the application of organic manure, which introduces into the soil certain organic compounds possessing the property of base exchange in a very high degree.

The other limed plots are almost equal in their amounts of exchangeable calcium.

In most cases, the exchangeable calcium for the different plots is found to be approximately 80 per cent of the total base exchange capacity. The relatively high percentage of exchangeable calcium in plot 1B is related to the higher content of calcium carbonate found in this soil.

Exchangeable magnesium

In table 2 are given the results for the exchangeable magnesium. The content of this base in the exchange complex varies from 3.8 per cent to 12 per

cent. The highest content of exchangeable magnesium, like that of exchangeable calcium is in plot 5B.

Calcium carbonate in the limed plots

In the last column of table 1 is given the calcium carbonate content of the limed soils. The results indicate that the amounts of calcium carbonate differ for the different plots in spite of equal applications of lime to all the plots. With the exception of 10B, in all plots to which muriate of potash was applied, the content of calcium carbonate is lower than in the check plot 1B and in plot 3B, which received only superphosphate. The potassium chloride has a solubility effect not only on the exchangeable calcium, as has been pointed out, but also on carbonate of calcium. The action of muriate of potash on calcium

TABLE 4

Amount of calcium adsorbed in the unlimed and limed soils, after equilibrium in the calcium carbonate paste

UNLIMED					LIMED				
Plot no.	CaO after equilibrium in the $CaCO_3$ paste		CaO adsorbed		Plot no.	CaO after equilibrium in the $CaCO_3$ paste		CaO adsorbed	
	Per cent	Milliequivalents in 100 gm. soil	Per cent	Milliequivalents in 100 gm. soil		Per cent	Milliequivalents in 100 gm. soil	Per cent	Milliequivalents in 100 gm. soil
1A	0.110	3.92	0.052	1.87	1B	0.144	5.15	0.003	0.12
2A	0.106	3.78	0.067	2.38	2B	0.135	4.85	0.025	0.87
3A	0.139	4.96	0.065	2.31	3B	0.145	5.19	0.010	0.35
4A	0.127	4.54	0.064	2.29	4B	0.158	5.64	0.014	0.49
5A	0.137	4.88	0.062	2.20	5B	0.207	7.40	0.018	0.63
9A	0.132	4.74	0.058	2.08	9B	0.137	4.91	0.012	0.43
10A	0.134	4.77	0.060	2.14	10B	0.150	5.35	0.014	0.49
11A	0.114	4.06	0.102	3.66	11B	0.143	5.13	0.034	1.23
12A	0.122	4.36	0.048	1.70	12B	0.155	5.55	0.013	0.46

carbonate was observed by Goessman (8), who pointed out that common salt and muriate of potash decompose the carbonates of lime and magnesia of the soil, changing those carbonates from a comparatively insoluble form to a soluble salt, which is carried out from the soils in the drainage water.

UNSATURATION OF THE SOIL

The exchangeable complex of the soils is found to contain not only metallic ions, but also hydrogen ions. The soils, the exchangeable complex of which contains a certain amount of hydrogen ions, are called "unsaturated" as compared with "saturated," which yield only metallic cations in the exchange reaction (6).

The introduction of fertilizers changes markedly the state of saturation of the soil exchange complex; the amounts of the metallic ions can be increased

or reduced by application of the various fertilizers, depending on the nature of the last ones.

The soils of the unlimed A and limed B plots, which were treated for many years with different kinds of fertilizers, differ greatly in their state of saturation. The unsaturation values of the soils from plots A and B are presented in table 4. The figures represent the amounts of hydrogen ion in the exchangeable complex that were replaced by the calcium when brought into contact with calcium carbonate paste, according to the Mattson method, and thus characterizing the unsaturation of the different soils.

The highest unsaturation is found in plots A. These soils adsorbed great amounts of calcium from the paste, which approached the amounts of exchange-

TABLE 5

Degree of unsaturation in the unlimed and limed soils

UNLIMED				LIMED			
Plot no.	Total base exchange capacity in mgm. equiv. in 100 gm. soil	Unsaturation	pH	Plot no.	Total base exchange capacity in mgm. equiv. in 100 gm. soil	Unsaturation	pH
		per cent				*per cent*	
1A	4.50	41.5	5.03	1B	5.69	2.1	7.62
2A	4.60	51.7	5.03	2B	5.50	15.8	7.33
3A	5.28	43.6	5.04	3B	6.03	5.7	7.39
4A	4.89	46.9	5.05	4B	6.40	7.6	7.20
5A	5.56	39.5	5.12	5B	8.39	7.6	6.95
9A	5.21	39.9	5.47	9B	5.74	7.5	7.26
10A	5.03	42.3	5.37	10B	5.91	8.3	7.40
11A	4.61	79.4	3.90	11B	5.68	21.6	7.13
12A	4.44	38.3	5.57	12B	6.49	7.1	7.26

able calcium present in the soils and in some cases surpassed them. The degree of unsaturation is given in table 5 as the percentage of the exchangeable hydrogen in relation to the total base exchange capacity of the soil. The degree of unsaturation is calculated according to the following formula:

$$\frac{\text{Calcium adsorbed by 100 gm. of soil in mgm. equiv.} \times 100}{\text{Total base exchange capacity in 100 gm. of soil in mgm. equiv.}}$$

Unsaturation in the unlimed plots A

The degree of unsaturation for the A plots fluctuates from 38 per cent to 79 per cent depending upon the application of the different fertilizers. Figure 2 represents graphically the degree of unsaturation for the various A plots. The most unsaturated plots are found to be 2A and 11A. The muriate of potash and ammonium sulfate, producing the loss of exchangeable calcium in these did not substitute their basis for the calcium lost: The calcium removed is mostly found to be replaced by hydrogen.

As has been indicated, the effect of ammonium sulfate in the removal of the bases is due mostly to the action of the acids formed and the consequent introduction of hydrogen ions in the exchangeable complex. With muriate of potash, there is at first a replacement of other bases by the potassium ion and a subsequent loss of this base by replacement with hydrogen. The exchangeable complex saturated with monovalent bases is less stable when combined with these bases than in the case of divalent ones. Such a complex is more readily attacked by water, and as a result of this water action, the monovalent bases are more easily removed.

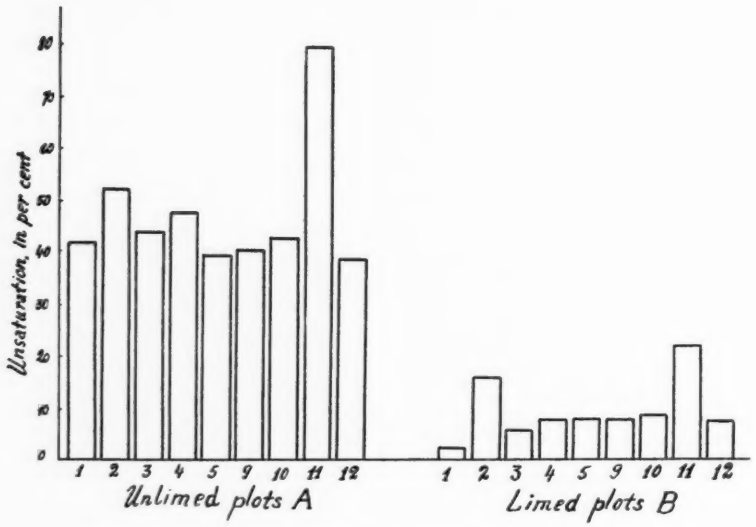

FIG. 2. DEGREE OF UNSATURATION IN THE UNLIMED AND LIMED SOILS

The exchangeable potassium can be removed also by the assimilation of plants. Martin (17) and Fraps (5), in recent papers concerning the effect of crop growth on the exchangeable potassium, show that the plants remove considerable amounts of exchangeable potassium from the soil.

Unsaturation in the limed plots B

Plots B (table 4 and fig. 2) likewise were found unsaturated to a certain degree. It can be noted that in spite of the presence of calcium carbonate in the soil all the plots contain a certain amount of hydrogen in the exchangeable complex. The presence of the replaceable hydrogen in the limed plot 11B, was previously observed by Joffe and McLean (2).

Because of many factors (such as the percolation of water through the soil,

bacterial activities, growth of plants, the effect of fertilizers), the complete saturation is evidently not reached under field conditions in the presence of small amounts of calcium carbonate. The greatest unsaturation is found in plots 2B and 11B, which is 15.8 per cent and 21.6 per cent, respectively. The unsaturation for the other B plots, with the exception of the check plot 1B, varies only between 5.7 per cent and 8.3 per cent.

LIME REQUIREMENT OF THE SOILS

In these experiments, the "lime requirement" means the amount of calcium adsorbed in the presence of unlimited quantities of calcium carbonate. This amount was determined, according to the Mattson method, by bringing the unsaturated sample of soil into contact with the calcium carbonate paste until equilibrium between the calcium carbonate and the soil was established. This

TABLE 6

"Lime requirement" (calcium oxide) for the unlimed and limed soils, according to Mattson's method

PLOT NO.	LIME REQUIREMENT (CaO) POUNDS AN ACRE	PLOT NO.	LIME REQUIREMENT (CaO) POUNDS AN ACRE
1A	1,040	1B	60
2A	1,340	2B	500
3A	1,300	3B	200
4A	1,280	4B	280
5A	1,240	5B	360
9A	1,160	9B	240
10A	1,200	10B	280
11A	2,040	11B	680
12A	960	12B	260

means of determining the lime requirement approaches more closely the natural conditions of lime application.

The rapidity and completeness of saturation will be dependent on the concentration of calcium hydroxide in the soil solution which appears as the result of the hydrolysis of the calcium carbonate. The liming under field conditions will approach the results of the laboratory experiments, and a more rapid and complete saturation of the exchangeable complex will depend on the introduction of somewhat increased amounts of lime, based on the amount found necessary by the laboratory paste method; the fineness of the lime applied; and the careful mechanical mixing of the soil after the introduction of lime.

Table 6 gives the amounts of lime, as calculated by Mattson's method, necessary for complete saturation of the soil of plots A and B. The figures are given for an acre, to a depth of $6\frac{3}{4}$ inches, on the basis of 2,000,000 pounds of soil.

COMPARISON OF LIME REQUIREMENT AND pH VALUE IN THE SOILS

Many experiments were carried out to compare the lime requirement with the pH value in an attempt to establish a correlation between the two values. Often, soils having the same pH values are found to vary in their lime requirement. Christensen and Jensen (4), investigating a number of soils having the same pH value, found a great difference in the amount of lime required for neutralization. Johnson (11) points out that there is no relation between the lime requirement and the hydrogen-ion concentration. Joffe and McLean (12) showed no correlation to exist between the pH value and the total replaceable hydrogen in the complex. Other investigators, however, found a correlation

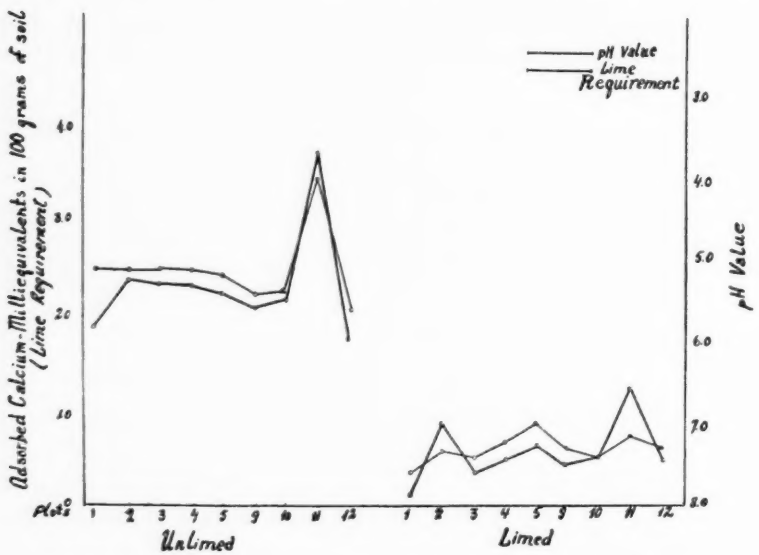

FIG. 3. RELATIONSHIP BETWEEN THE "LIME REQUIREMENT" AND THE pH VALUES

between the two values. Blair and Prince (2) found a fairly close correlation between the H-ion concentration and the lime requirement. Some correlation has been obtained by Carleton (3) for the hydrogen-ion concentration and lime requirement for soils of the same type.

Ogg and Dow (24) showed that heavy soils had a higher lime requirement than light soils.

The relationship between the two values depends chiefly on the buffer and adsorption capacity of the different types of soil. Mattson (19, 20) showed that the colloidal material of different soils varies greatly in base adsorption power according to their composition ratio of silica/sesquioxides. The buffer and exchange capacity increases with an increase in this ratio.

In the case of the Sassafras soils with different lime requirements for various plots which belong to the same type of soil and have almost the same physical and chemical composition for each section, an opportunity was given to find whether a relationship exists between the lime requirement and the pH value. The results obtained (tables 5 and 6) for the lime requirement and for the reaction of all the unlimed and limed plots show that there is a certain correlation between these two values. The reaction of the plots unsaturated to different degrees thus reflects the state of their unsaturation. The correlation is graphically represented in figure 3.

The reaction of the soils

Table 6 gives the pH value of the unlimed A and limed B plots. The pH of the A plots varies from 3.9 to 5.57. The pH for all the limed plots is higher, the reaction being alkaline in all plots with the exception of 5B. Plots 2B and 11B, of which the degree of unsaturation rises to 15.8 and 21.6 per cent respectively, are slightly alkaline. Thus it is seen that the soils are unsaturated with respect to their reaction with calcium carbonate and still have an alkaline reaction. The calcium-magnesium-sodium-potassium-hydrogen complex is analogous to the salts of the sodium phosphate, which may contain displaceable hydrogen and not be acid. Similarly, the reaction of a soil may be alkaline and yet react with calcium carbonate.

From the foregoing discussion it is seen that the reaction reflects relatively the state of unsaturation of the soil studied, but does not show the actual degree of base saturation of the exchange complex.

SUMMARY

Some of the methods applied were studied in detail and discussed. Mattson's calcium carbonate paste method was studied in detail before its application to the determination of lime requirement. Hissink's, Gedroiz's, and Tyurin's methods for the determination of the calcium carbonate dissolved by the sodium chloride solution, were compared. Hissink's correction for the dissolved calcium carbonate was found to be unsatisfactory for the limed Sassafras soils. Gedroiz's and Tyurin's methods were found to give almost identical results for the amount of calcium carbonate dissolved.

The effect of different fertilizer treatments on the content of exchangeable calcium and magnesium, the degree of unsaturation, the lime requirement, and the reaction were studied. The amount of exchangeable calcium is found to vary with the kind of fertilizer applied to the soil. The calcium carbonate in all cases increased considerably the amount of exchangeable calcium. The superphosphate increased to a certain degree the amount of the exchangeable calcium. The muriate of potash is found to remove the exchangeable calcium both in the unlimed and limed soils. The ammonium sulfate appeared to have the most exhaustive action in removing the exchangeable calcium from the soil. The greatest effect was produced on the unlimed soil. The application of manure increased the total base exchange capacity of the soils and in the

limed plot, the amount of the exchangeable calcium. The unlimed soils were found to be highly unsaturated, the degree of unsaturation being influenced by the fertilizer applied. A correlation was found between the lime requirement and the pH value, in both the unlimed and the limed soils.

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A CRITICAL STUDY OF THE METHODS FOR DETERMINING THE NATURE AND ABUNDANCE OF SOIL ORGANIC MATTER[1]

SELMAN A. WAKSMAN AND KENNETH R. STEVENS

New Jersey Agricultural Experiment Station

Received for publication January 3, 1930

Among the numerous problems dealing with soil organic matter, which is complicated both in nature and origin, none has received more attention than the methods suggested for its quantitative determination. These methods have been concerned either with the determination of the soil organic matter as a whole or only with a certain definite fraction that has been commonly considered to be of the greatest importance in soil fertility. The term "humus" is usually applied indiscriminately both to the total organic matter and to some portion of it which has become an integral part of the soil.

METHODS FOR DETERMINATING TOTAL SOIL ORGANIC MATTER

It is now generally agreed that the determination of the total organic matter content of the soil by the loss on ignition is hardly a fair measure, since the chemically combined water is also included in this determination. The common error found in the use of this method may range from 10 per cent, in the case of sandy soils, to 50 or even 70 per cent, in the case of clay soils. This unusually large error makes the determination totally unacceptable for accurate work.

The other common method available for this purpose is the determination of the total carbon content of the soil; this can be obtained by one of the dry or one of the wet combustion methods. The amount of organic carbon found in a definite quantity of soil is then multiplied by the factor 1.724 to give the total content of soil organic matter. This factor has been obtained by Van Bemmelen, Wollny, and other soil chemists, on the assumption that the carbon content of soil organic matter is 58 per cent. It will be brought out in the following pages that this assumption was fairly accurate and that this method is still the most reliable that we have at the present time for measuring the total organic matter of the soil. If the soil contains carbonates, these should be determined separately and correction made on the total carbon found in the given soil.

Because the carbon-nitrogen ratio of the soil organic matter is usually found to be about 10 to 1, it has been suggested that the organic matter content of

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

the soil be calculated from the total nitrogen found (1), namely, by multiplying the nitrogen figure by 20. This is often far from accurate, however, because the ratio (C:N) is frequently found to be much narrower than 10:1, in which case an abnormally high organic matter content will be reported.

Various modifications of the total carbon method have been suggested, based upon the oxidation of the organic matter, whereby either the amount of oxidizing agent consumed, as in the chromic acid method of Schollenberger (13), or the amount of reduction brought about, as in the sulfur dioxide method of Robinson (9), is determined. These methods may prove convenient for practical purposes where a rapid determination of the approximate concentration of organic matter in the soil is desired. The results will be invalidated, however, when the soil is rich in reducing substances of an inorganic nature. The use of a 30 per cent solution of hydrogen peroxide for the complete oxidation of the total soil organic matter can also be grouped among the methods for determinating total carbon in soil (10).

For a careful qualitative and quantitative study of the soil organic matter the determination of total carbon remains the most reliable, provided the nature of this organic matter is understood.

DETERMINATING DEFINITE FRACTIONS OF SOIL ORGANIC MATTER

In addition to determining total organic matter in the soil, numerous attempts have been made to analyze for certain definite organic fractions that were believed to play specific functions in the physical, chemical, or biological soil processes. The analysis of these fractions was based upon the old, totally unjustified assumption of Hilgard and others that plant residues added to the soil must be "humified" before the nutrient elements can become available for plant utilization. In other words, the "humified" organic matter was considered to be the intermediary material between the plant remains that have been introduced into the soil in the form of roots, stubble, green manures, and stable manures, and the final products of decomposition, such as ammonia and carbon dioxide. The complete lack of justification for this assumption has been amply illustrated in a previous contribution (18) in which the literature on the origin of the so-called soil "humus" has been reviewed in detail. The unwarranted conclusions seemed to have found support in the fact that in the decomposition of plant and animal residues in the soil various dark colored substances, possessing definite physical and chemical properties are produced. However, these dark colored substances can hardly be considered as intermediary decomposition products; they should rather be looked upon as substances resulting from direct or indirect decomposition of plant residues that have resisted further degradation as well as of products of microbial synthesis.

It is interesting to note, in this connection, that the soil chemists and agronomists have considered this "humified" organic matter largely from the point of view of its nitrogen content. The chemists, in their study of the nature of soil "humus," frequently left the nitrogen out of consideration altogether,

since the formulas suggested for the various "humic acids" were entirely free from nitrogen, although no treatment with strong alkalies or acids could remove this nitrogen completely without destroying the whole complex.

The various methods which have been suggested at different times for determining this "humified" portion of soil organic matter can be conveniently divided into two groups:

1. Methods based upon the use of alkalies for the extraction of the soil "humus." This "humus" or "humic acid" is either precipitated from the alkali solution by an acid, or, when ammonia has been used for extraction, the solution is evaporated leaving the "humus" residue. In all these determinations—including that of the *matière noire* of Grandeau, "humic" and "hymetomelanic" acid of Hoppe-Seyler and Oden, and the α-fraction of the senior author—only a part of the soil organic matter, rarely more than 30 to 50 per cent, is usually obtained. The same thing is true of determinations based upon the depth of color of the alkali extract when compared with the color of an alkaline solution of a standard "humic acid" preparation, or some definitely colored solution. Because the chemical identity of this fraction is still a matter of discussion and because varying quantitative results were obtained depending on the nature and concentration of the alkali, on the temperature and length of extraction, and on the nature of precipitating agents (17, 19), this method may be abandoned for any accurate measurement of soil organic matter or of any definite fraction of it.

2. The oxidation of the "humified" portion of the soil organic matter by mild oxidizing agents, such as dilute hydrogen peroxide, permanganate solution, and hypochlorite solution. The value of all of these methods has been questioned, especially in view of the fact that we know nothing of the nature of the organic complexes which are acted upon by these reagents, as is shown later.

These two methods are based upon two distinctly different properties of a certain part of the organic matter, namely, solubility in alkalies and oxidation by certain weak oxidizing agents. Nothing definite is known concerning the chemical nature of these complexes; it is not known whether the two groups of methods deal with the same or with different chemical substances.

Springer, who reviewed these methods in detail recently (17), made an exception for the acetyl bromide method of Karrer. This reagent was found to dissolve natural plant constituents, but it leaves the so-called "humus" or newly formed, dark-colored substances intact. The method may yield interesting information in the study of decomposition of plant materials in the compost, such as in the differentiation between certain residual and resistant plant constituents and modified or synthesized complexes, but we can hardly expect that it may serve as a general method for determining the nature and abundance of soil organic matter.

For the purpose of throwing a certain amount of light upon the oxidation methods as a means of determining the nature of the particular organic complexes which are thereby oxidized, and of determining whether the substances thereby oxidized have any definite relation to the so-called "humus" complexes in the soil, the following experiments were carried out: Four preparations; namely, finely ground fresh chestnut wood, wood rotted by brown rot fungi and resulting in a product which consisted of 71 per cent lignin and modified

lignin complexes, forest soil consisting of surface material (F-layer) of a spruce forest, and lowmoor (sawgrass) peat from the Everglades, Florida. A detailed chemical analysis of the four materials is given in table 1.

Portions of the air-dried and ground materials were treated with a 6 per cent hydrogen peroxide solution, according to Robinson's method (8), and with a solution of chlorine dioxide (ClO_2), according to the method of Schmidt (11). The last method is known to dissolve all lignin in plant material and has found considerable application in plant chemistry.

TABLE 1

Chemical composition of four organic substances used for oxidation studies

In per cent of total dry material

CHEMICAL COMPLEXES	SOUND CHESTNUT WOOD	ROTTED WOOD	FOREST SOIL (F-LAYER)	LOWMOOR PEAT
Ether-soluble	2.66	1.48	3.58	2.98
Cold- and hot-water-soluble	7.08	1.26	5.14	1.73
Alcohol-soluble	3.27	5.05	.....	1.06
Hemicelluloses	15.23	4.72	17.50	6.41
Cellulose	23.58	2.16	9.62	0.28
Lignins	22.05	71.14	42.26	46.12
Protein	0.54	1.31	6.84	23.06
Ash	0.54	0.65	6.05	10.00

TABLE 2

Influence of oxidation of fresh and decomposed plant residues with a six per cent solution of hydrogen peroxide and with a solution of chlorine dioxide

NATURE OF MATERIAL	PROPORTION OXIDIZED BY	
	H_2O_2	ClO_2
	per cent	*per cent*
Sound chestnut wood	20.1	28.0
Rotted wood	61.4	92.5
Forest soil	67.6	45.2
Lowmoor peat soil	80.2	73.0

Table 2 shows that a 6 per cent solution of hydrogen peroxide is capable of oxidizing not only decomposed or "humified" organic material but also a certain amount of fresh plant material; actually 20 per cent of the total sound chestnut wood was oxidized by this reagent. If that is the case, how much emphasis can be laid upon the determination of the so-called "humified" matter even in decomposed material by the use of this method? The rotted wood which contains 75 per cent lignin, as shown both by direct lignin determinations and by treatment with chlorine dioxide, is oxidized by hydrogen peroxide to the extent of 61 per cent. The lowmoor peat, which contains 10 per cent ash, 23 per cent nitrogenous compounds, various ether- and alcohol-soluble substances, hemicelluloses, and but 46 per cent of lignins and modified

lignin ("humus") complexes, was oxidized to the extent of 80 per cent by the peroxide reagent. One would hesitate to apply the term "humified" material to these 80 per cent of peat constituents, since a large part of it is no doubt of plant origin and since the term itself is meaningless in any attempt to interpret the chemical nature of the constituents as well as the processes of peat formation.

The chlorine dioxide reagent also reacts with various plant constituents, oxidizing more than the lignin fraction in the organic material. This is especially well brought out in the data on the sound chestnut wood and rotted wood. Here as well, it is rather difficult to interpret the results obtained by this method in terms of definite constituents of the decomposed plant residues. One would hardly be justified in any attempt to apply these results to an interpretation of the nature of the organic matter in soils and peats.

For the purpose of interpreting further the nature of those chemical constituents of the plant residues and of the decomposed organic matter which are acted upon by these oxidizing agents, definite quantities of the aforementioned four organic substances were treated with ether, then with hot alcohol, and finally with hot water. Various fractions of the four preparations thus extracted were now divided into several series and treated as follows: (*a*) One set was oxidized directly with hydrogen peroxide and chlorine dioxide reagents and the amount of material thereby lost determined. (*b*) Another set was boiled with 2 per cent hydrochloric acid, at 100°C. for 5 hours, to remove all the hemicelluloses. The residues were filtered, washed thoroughly with distilled water, and treated with the two oxidizing agents. (*c*) A third set of samples was treated with hydrochloric acid as in set (*b*), filtered, washed, dried, and then further treated with 10 volumes of 80 per cent sulfuric acid for 2 hours; this was then diluted with 15 volumes of water and boiled for 5 hours, the process resulting in a complete removal of the celluloses. The material left after this treatment consists of lignin with a small admixture of protein and ash; it was again subjected to the action of the two oxidizing agents. The data obtained as a result of the oxidation of these various preparations were calculated back to the original material and reported on a percentage basis (table 3).

From the results on the oxidation of the chestnut wood preparations by chlorine dioxide, one may conclude that this reagent acts primarily upon the lignin and to some extent upon the hemicelluloses of the plant tissues. With an increase in the lignin content of the preparation there is an increase in the amount of material removed by the chlorine dioxide reagent; finally when pure lignin is left, as a result of the acid treatment, 99.4 per cent of the material is oxidized by chlorine dioxide. However, the 6 per cent hydrogen peroxide solution acts only to a limited extent upon several of the plant constituents, including some of the lignin and some of the water-and alcohol-soluble constituents.

The rotted wood contained 71 per cent lignin, and one would, therefore, expect that it should be acted upon readily by the chlorine dioxide reagent; this

was actually found to be the case. The hydrogen peroxide reagent acted only partly upon the lignins; it seemed to have exerted a marked effect upon the non-lignin constituents of the wood; namely, the synthesized cell substance, the alcohol-soluble substances, and the hemicelluloses.

TABLE 3

The action of hydrogen peroxide and chlorine dioxide upon different chemical constituents of fresh and decomposed plant materials

On per cent basis of original material

NATURE OF MATERIAL	TREATMENT OF PREPARATION	PROPORTION OXIDIZED BY	
		H_2O_2	ClO_2
		per cent	*per cent*
Chestnut wood	Untreated	20.1	28.0
	Freed from ether-, alcohol-, and hot-water-soluble fractions	14.1	26.1
	Freed from above fractions and hemicelluloses	9.8	45.3
	Freed from above fractions and celluloses	13.0	99.4
Rotted wood	Untreated	61.4	92.5
	Freed from ether-, alcohol-, and hot-water-soluble fractions	54.8	94.4
	Freed from above fractions and hemicelluloses	32.5	92.5
	Freed from above fractions and celluloses	14.7	99.7
Forest soil	Untreated	67.6	45.2
	Freed from ether-, alcohol, and hot-water-soluble fractions	66.4	47.7
	Freed from above fractions and hemicelluloses	46.2	48.0
	Freed from above fractions and celluloses	31.9*	87.6*
Peat soil	Untreated	80.2	73.0
	Freed from ether-, alcohol-, and hot-water-soluble fractions	83.8	74.3
	Freed from above fractions and hemicelluloses	81.1	76.1
	Freed from above fractions and celluloses	53.4*	82.7*

* Results calculated on basis of ash-free residue.

The data dealing with the various preparations of the forest soil and peat are very illustrative. Results of the chlorine dioxide treatment indicate definitely that the lignin-like complexes, as determined by the method of analysis of forest and peat soils (22, 23), are actually lignin in nature. The removal of the

alcohol-soluble fraction, of the hemicelluloses, and of the celluloses increases the lignin content of the preparation and also the amount of material acted upon by the chlorine dioxide. However, with the hydrogen peroxide reagent, the maximum effect was exerted upon the untreated material and upon the preparation treated with ether, alcohol, and hot water, whereas a minimum effect was exerted upon the lignin residues. If we assume that 6 per cent hydrogen peroxide acts upon the "humified" or transformed portion of the plant material, then we must assume that this "humus" consists also of fats and waxes, of pectins, hemicelluloses, and even of celluloses and lignins.

It is interesting to note in this connection that acid lignin prepared from chestnut wood was acted upon completely by chlorine dioxide, as one would expect, and only to the extent of 10.5 per cent by the hydrogen peroxide reagent, whereas alkali lignin obtained from the same wood (extracted by 4 per cent sodium hydroxide solution under pressure, then precipitated with hydrochloric acid) was acted upon completely by chlorine dioxide and to the extent of 32.3 per cent by the 6 per cent hydrogen perioxide. In other words, the action of the 6 per cent hydrogen peroxide depends upon the chemical structure of the plant constituent as well as upon the method used for obtaining it. When one considers further that the fact that this reagent brought into solution 44.6 per cent of the α-fraction ("humic acid") of a light loam soil (Sassafras) and 90.5 per cent of the α-fraction obtained from a peat soil, one would hardly want to venture any suggestion in regard to the degree of "humification" or degree of decomposition of either of these preparations or of the materials from which they were obtained, when measured by the hydrogen peroxide reagent.

The use both of alkalies and of such oxidizing agents as dilute hydrogen peroxide offers, therefore, little promise for determining either the abundance, nature, or degree of decomposition of the soil organic matter. Gortner (2) several years ago showed that as far as the use of alkalies for determining and isolating the soil "humus" is concerned, one is able to obtain the same type of "humus" from fresh plant materials as from soil. Of course, the substance that he actually isolated from the plant material consisted of lignin with perhaps some admixture of hemicelluloses; he referred to this complex as "humus," merely because the same method that was commonly employed for obtaining the soil "humus" was used in its preparation.

CHEMICAL NATURE OF SOIL ORGANIC MATTER

The question may then be asked: What should be the approach to a quantitative and qualitative study of the nature of the soil organic matter? We must of course differentiate in this connection the following three distinct problems: 1. The quantitative determination of the total organic matter in the soil, which, as shown in the foregoing, can at present be best calculated from the total organic carbon in the soil. 2. A knowledge of the degree of decomposition of

organic matter in the soil; however, to be able to study the various stages of decomposition of soil organic matter we must first gain a proper understanding of the mechanism of the decomposition processes; these are still imperfectly understood. 3. A study of the chemical composition of the soil organic matter; this has already been attempted by numerous investigators, and any new method of approach can only add further information to this complicated problem.

An attempt has already been made (21, 24) to apply the proximate system of analysis, which has been developed for the study of the processes of decomposition of plant materials, to the analysis of peat and forest soils, which are largely organic in nature. By this system of analysis, as much as 80 to 95 per cent of the constituents of these two organic soil formations have been accounted for in terms of definite chemical complexes.

One may now go a step further and apply this system of analysis to the study of the organic matter in inorganic soils. The method, of course, would have to be considerably modified, since one is dealing here with a mass of material containing only 1 to 10 per cent of organic matter and 90 to 99 per cent of inorganic constituents.

Keppeler (3) suggested that peat be treated with concentrated sulfuric acid solution (72 per cent), then the amount of reducing sugar produced be determined; the latter was found to serve as a good index of the degree of decomposition of peat: the less reducing sugar formed the more the peat is decomposed. Unfortunately, the fact was not considered that lowmoor, highmoor, and sedimentary peats are derived from different plant residues varying markedly in composition: although each can be compared with other peats of the same nature, they cannot be compared with one another, as has been shown elsewhere (22).

The carbon, nitrogen, and moisture contents of the untreated soil are determined on separate portions. The complete analysis can be carried out as follows: Two 100-gm. portions of air-dry soil are extracted first with ether in Soxhlets, for 12 to 24 hours. The ether extract is evaporated to a small volume, transferred to weighing bottles, dried to constant weight, and the amount of ether-soluble material determined. The ether-treated soil is next extracted with hot 95 per cent alcohol for 1 to 2 hours, on a water bath. The alcohol-soluble material is determined in the extract. The residual soil may then be treated with hot water; this is followed by extraction with 2 per cent hydrochloric acid solution, for 5 hours at 100°C.; the hot-water extract may be omitted and the soil treated with the acid following the alcohol extraction. The acid extract is filtered off and the soil washed with distilled water. The filtrate and washings are combined and made up to volume. One aliquot portion is used for a determination of reducing sugar, one for total nitrogen, and one for ammonia-nitrogen determinations. The total amount of organic matter that has gone into solution may be calculated from the carbon content in an aliquot portion of the extract. The sugar and the ammonia can serve as

indexes of the hemicellulose and amide content of the organic matter in the particular soil.

It should be noted here that after the soil has been treated with hydrochloric or with sulfuric acid, a solution is obtained which is frequently very rich in iron and aluminum. These elements interfere with an accurate sugar determination. After neutralization, the iron and aluminum precipitate should be filtered off, and the residue washed; the sugar is then determined in the filtrate. One must make sure that the results check well.

Aliquot portions (20 to 50 gm.) of the dry residue left after the hydrochloric acid treatment are now placed in flasks and 20 to 30 cc. of 80 per cent sulfuric acid solution is added. The acid is allowed to act upon the soil for 2½ hours in the cold. The material is then diluted with 15 volumes of water (300 to 450 cc.) and boiled for 2 to 5 hours. This treatment results in the complete hydrolysis of the cellulose found in the soil and its transformation into glucose. The determination of the reducing sugar can serve as a fair index of the cellulose content of the soil. The residue from the sulfuric acid treatment is thoroughly washed with water, dried, weighed, and analyzed for total carbon and nitrogen. This residual organic matter consists of lignin and its transformation products and of various synthesized microbial complexes including certain organic nitrogen compounds. If one assumes that the distribution of the nitrogen in the organic nitrogenous soil complexes is the same as the nitrogen distribution in native plant and animal proteins, one can calculate the amount of organic nitrogenous substance and the residual material by multiplying the nitrogen content by the factor 6.25.[2] By subtracting from the total carbon found in the residue the carbon of the nitrogenous complex, assuming that the latter contains 50 per cent carbon and allowing 62 per cent carbon in the "lignin-humus" complex, one can calculate the amount of the "lignin-humus" complex in the residual soil and in the original soil. The following formula may be used for this purpose:

$$\text{Per cent of "lignin-humus" complex in soil} = \frac{a \times 100}{A} - \frac{b \times 100}{S},$$

where a = the carbon content in the sulfuric acid residue, calculated on the basis of the total original sample of soil,

A = the total carbon content of the sample of soil,

b = protein content in the sulfuric acid residue, obtained by multiplying the nitrogen content of the residue by 6.25; this is then calculated for the whole sample,

S = total organic matter in the soil sample, as calculated from the organic carbon of the soil.

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[2] The use of the factor 6.25 for calculating protein or organic nitrogenous compounds from the total nitrogen of the soil may prove to be far from accurate. Before any more definite information has been obtained concerning the chemical nature of the nitrogenous compounds of the soil, the factor should be looked upon as tentative. It should be emphasized here as well that the factor 1.72 for determining the total organic matter, from the carbon content should also be considered as merely tentative.

It may prove desirable to subdivide this "lignin-humus" complex further into certain subgroups; this can be accomplished by the use of a reagent like acetyl-bromide, by the solubility of a part of the complex in ammonium hydroxide solution, or by the formation of a precipitate as a result of the acidification of the hot sodium hydroxide extract of the material.

For carrying out this analysis, several soils from different parts of the United States and Canada have been used. These soils can be described as follows:

Soil no. 4. Summit soil from Missouri, Hor. A.
Soil no. 6. Chernozem soil from Hays, Kansas, Hor. A.
Soil no. 10. Alpine humus, from Pike's Peak, at a height of 13,800 feet.
Soil no. 16. Chernozem soil from Edmonton, Alberta, Canada, taken at a depth of 1 to 25 cm.
Soil no. 18. Brown soil at Indian Head, Saskatchewan, Canada, depth 1 to 20 cm.
Soil no. 21. Chernozem soil from Brandon, Mannitoba, Canada, depth 1 to 20 cm.
Soil no. 22. Same as no. 21, 25 to 50 cm. depth.
Soil no. 29. Carrington loam, dark colored, prairie soil, Hor. A.

A summary of the carbon, nitrogen, and organic matter content of these soils is given in table 4. These results show, first, that there is a considerable discrepancy in the organic matter content of the soil as determined by loss on ignition and as calculated from the carbon content. The loss on ignition is usually 35 to 75 per cent higher than the organic matter calculated from the carbon content and, in some cases even 118 per cent higher. Secondly, the fairly uniform ratio between the carbon and nitrogen of the soil is of considerable interest. This ratio ranges within narrow limits between 9.9 to 12.0.

Table 5 gives the results of the proximate chemical composition of the organic matter found in these soils. These results were calculated on the basis of the total soil organic matter, the latter being determined from the total carbon figures given in table 4.

These results are highly interesting. They prove definitely that the chemical composition of organic matter in different soils varies considerably. This is true especially of the non-nitrogenous constituents. The fat and wax content (ether-soluble portion) varies from 0.46 to 4.71 per cent of the total organic matter. A similar variation is found in the amount of alcohol-soluble constituents (resinous substances). The celluloses and hemicelluloses in the soil also vary, although not to such an extent. It is interesting to note that the hemicellulose content of the soil organic matter is considerably larger than the cellulose content, just the reverse of what is found in plant residues. This is because the hemicellulose (including the pentosans and methyl-pentosans) group comprises also substances which have been largely synthesized by the soil microörganisms in the building up of their cell substance. It is interesting to note that some of the reducing sugars obtained in the cellulose fraction may represent not true plant cellulose but microbial cellulose or chitin.

The two largest groups of chemical complexes found in the soil organic

matter are the "lignin-humus" complex ("soil lignin" or "soil humus") and the nitrogenous compounds. These two groups make up 71 to 80 per cent of the total organic matter of the soil and an even larger amount of that part of the organic matter which is accounted for in these analyses. The high carbon content of the soil organic matter (usually taken as 58 per cent) is due to the predominance of the lignins, lignin-like complexes, and lignin derivatives with a high carbon content (62 to 64 per cent). The more or less definite relation

TABLE 4

The nature of the organic matter in different mineral soils

On the basis of dry soil

SOIL NO.	ORGANIC MATTER BY IGNITION	ORGANIC MATTER C × 1.72	TOTAL CARBON	TOTAL NITROGEN	C/N	pH OF SOIL
	per cent	*per cent*	*per cent*	*per cent*		
4	7.89	4.49	2.61	0.2417	10.8	6.82
6	5.98	2.74	1.59	0.1543	10.3	7.56
10	11.00	7.74	4.50	0.4429	10.1	5.08
16	17.10	11.20	6.51	0.6695	9.9	6.38
18	10.34	6.24	3.63	0.3324	10.9	8.26
21	10.06	7.40	4.30	0.3950	10.9	8.25
22	12.03	8.31	4.83	0.4403	11.0	8.26
29	10.16	6.48	3.77	0.3145	12.0	7.75

TABLE 5

Proximate chemical composition of soil organic matter

On the basis of the total soil organic matter (C × 1.72)

SOIL NO.	ETHER-SOLUBLE MATERIAL	ALCOHOL-SOLUBLE MATERIAL	HEMICELLULOSES	CELLULOSE	LIGNIN-HUMUS COMPLEX	ORGANIC NITROGENOUS COMPLEXES	SUM OF THE CONSTITUENTS ACCOUNTED FOR
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
4	3.56	0.58	5.44	3.55	43.37	33.78	90.28
6	4.71	1.53	8.60	5.22	40.81	34.74	95.61
10	1.94	3.10	12.59	5.36	35.18	35.77	93.94
16	0.80	0.82	5.53	4.12	41.87	37.35	90.49
18	1.02	0.88	6.96	3.50	42.05	33.25	87.66
21	0.46	0.84	8.54	2.83	42.83	33.36	88.86
22	0.52	0.63	10.66	3.38	46.50	33.13	94.82
29	0.62	0.61	8.21	3.64	49.29	30.38	92.75

between the "lignin-humus" complex and the organic nitrogenous complexes accounts for the more or less constant ratio between the carbon and the nitrogen in the soil organic matter.

The close correlation between these two groups of complexes in the soil organic matter may be a matter of chance or it may be due to the actual formation of a chemical compound between the two, whereby they are made more resistant to the action of the microörganisms. It should be recalled

in this connection that the peats, especially the lowmoors, which are more closely related in their hemicellulose and cellulose content to the soil organic matter, are richer in lignins and lower in nitrogenous compounds than the organic matter of the soil; this explains the considerably wider carbon-nitrogen ratio of the peat material than of the soil organic matter.

Special attention should be called to soil 10, or the Alpine soil. Because of the very high altitude and prevailing low temperature, the organic matter produced by the roots and by other residues of the Alpine vegetation is not decomposed as readily as in normal field soils with a more optimum temperature for the activities of soil microörganisms. The high cellulose and hemicellulose content, the high ether- and alcohol-soluble fractions, as well as the low "lignin-humus" content point to the fact that this soil still contains some plant residues undecomposed or in the process of active decomposition.

These results bring out definitely the fact that by the method of analysis proposed here one may be able to account for 90 per cent or more of the soil organic matter in the form of definite chemical compounds. Although the exact chemical identity of some of the compounds may be questioned, still there is no room here for the application of such indefinite terms as "humus," "humified," "humic matter," as well as of the numerous "humic acids," which have been confusing, both as to their nature and their origin, to the investigator who has attempted to contribute to our knowledge of the soil organic matter. This method of proximate analysis can certainly not be recommended for a routine study of the nature of soil organic matter but it enables us to investigate the exact chemical identity of the constituents of the organic matter of the soil, just as a proximate analysis of plant material allows us to make a closer study of the nature of the chemical complexes in the plant which are still unknown.

The advantage of dividing the soil organic matter into several groups by the method proposed here over the alkali methods previously employed can hardly be questioned. Whatever the nature of the fatty, waxy, and resinous substances present (15) in various soils under different conditions of treatment, their grouping together into the ether- and alcohol-soluble fractions allows us to think of them in definite chemical terms. Whatever the nature of the hemicelluloses, whether they are pentosans and methyl pentosans, the presence of which has been demonstrated in soil by Michelet and Sebelien [about 4.2 per cent of the organic matter (6)], by König and associates (4), by Schreiner and Shorey (14), and by others, or hexosans such as galactans and mannans, or whether the sugar is obtained by hydrolysis of nucleo-proteids, oxycelluloses, pectins, and various uronic acid compounds, their classification into one group places them conveniently for further investigation. The same thing is true of the celluloses. Soil no. 16, for example, which is reported to contain 5.53 per cent hemicellulose and 4.12 per cent cellulose (on the basis of the soil organic matter), gave 6.85 per cent furfural-yielding substances, on distillation with 12 per cent hydrochloric acid. The two remaining groups, namely,

the organic nitrogenous compounds, which can be referred to as the "protein group," and the lignins or lignin-like complexes and modified lignins, which can be referred to as the "lignin" or the "lignin-humus" group, comprise 70 to 80 per cent of the total soil organic matter. The "protein" group, on hydrolysis with acids and alkalies, gives various amino acids, acid amides, and heterocyclic compounds. The relative amide content is considerably greater than in plant or animal proteins, but the nature of most of the heterocyclic compounds as well as of the melanoid-like compounds (12), still remains to be determined. The presence of the lignins and lignin-like complexes in soil can also be demonstrated by methoxyl determinations (6, 4, 16).

In all the past "humus" and "humic acid" determinations, when the soil was treated with an alkali solution at a high or low temperature for a short or a long period of time, several of the chemical complexes discussed here were acted upon; namely, the hemicelluloses, the "lignin" complex, and the "protein" groups; they were partly brought into solution and partly hydrolyzed depending upon the extracting agent and upon conditions of extraction. This action is very similar to that which results from the treatment of undecomposed plant material with an alkali solution, with the sole exception that, in the case of the soil, the problem is further complicated by the presence of a large amount of alumino-silicates which are also brought into solution by the alkali. When an acid is added to the alkaline soil extract a precipitate is formed, the amount and nature of which (such as nitrogen and ash content) depend upon the amount and nature of the precipitating agent and the temperature of precipitation. An exact similarity is found in the precipitation of the alkaline plant extract by acids. It is this precipitate which was termed "humic acid" and was frequently further separated on the basis of solubility in alcohol, in pyridine, and in other solvents. One can easily show that this "humic acid" is nothing but a mixture of: (*a*) certain hemicelluloses (in the broadest sense), since it gives reducing sugars on hydrolysis with dilute acids and furfural on distillation with 12 per cent hydrochloric acid; (*b*) nitrogenous organic compounds—since it usually contains 3 per cent nitrogen—part of which is readily hydrolyzed with dilute acids, giving amino acids and acid amides, and part of which cannot be hydrolyzed even by prolonged boiling with 20 per cent hydrochloric acid, pointing to its non-protein nature; (c) lignins and lignin-like complexes, as shown by its resistance to the action of 80 per cent sulfuric acid in the cold and by its methoxyl content. These phenomena will be discussed in detail in a later contribution.

One of the most important and interesting groups of chemical complexes in the proximate analysis of plant material, composted material, and soil organic matter is found in the "protein" group or the organic nitrogenous compounds. It should be recalled here that the percentage of "protein" in the soil organic matter is calculated by multiplying the total nitrogen of the soil (the water-soluble forms of nitrogen in ordinary field soil being quite negligible) by 6.25; the quantity thus obtained is divided by the total organic matter of the soil (carbon content x 1.72) and multiplied by 100.

One assumes thereby that the nitrogen content of the soil nitrogenous organic complexes is 16 per cent, which is probably far from accurate, and that the carbon content of the soil organic matter is 58 per cent, which is also somewhat too high, especially in view of the carbohydrate content of the organic matter. These figures must be reinterpreted with the further accumulation of our knowledge of the chemical composition of the soil organic matter.

Given a constant carbon-nitrogen ratio in the soil organic matter and constant factors for nitrogen and carbon, the percentage of "protein" in soil will be expected to be constant, as is actually the case. Should the C/N ratio always be 10, the theoretical amount of "protein" or hypothetical nitrogenous compounds in soil would be:

$$\frac{N}{C} \times \frac{\text{protein factor}}{\text{total organic matter factor}} \times 100 \text{ or } \frac{1}{10} \times \frac{6.25}{1.72} \times 100 = 36.34 \text{ per cent}$$

The "protein" content in soils 10 and 16 (table 5), which have a carbon-nitrogen ratio approaching the theoretical 10:1, more closely than that of the other soils, is actually found to be very close to this theoretical figure, namely 35.77 and 37.35 per cent. The wider the C/N ratio, the lower is the "protein" content of the soil organic matter; the narrower the ratio, the higher is the "protein" content.

The question then arises: What is the nature of these nitrogenous compounds? There is no doubt that they are partly protein-like in nature, because on hydrolysis with acids they give a large amount of various amino acids. However, the distribution of the amino groups in the proteins of the soil organic matter is quite different from that in the plant or animal proteins. As a matter of fact, Lathrop (5) and Morrow (7) have shown that no matter what the amino acid distribution in the proteins added to the soil, the newly formed soil "protein" is quite different in nature, and that different soils behave alike in producing the same type of protein. This points definitely to the fact expressed by the senior author repeatedly concerning the rôle of the microbial cell substance as a source of protein or of organic nitrogenous complexes in the soil organic matter.

The following experiment deals, in a preliminary manner, with the distribution of nitrogen in the various soils previously analyzed by repeated treatment with different concentrations of acid (table 6). Only between 22.5 and 33.5 per cent of the total soil nitrogen is made soluble by prolonged boiling with 2 per cent hydrochloric acid. The amount is increased to 38.4 per cent in the Alpine soil, which, as the general analysis shows, is still rich in undecomposed organic matter. Taking into consideration the average of all these soils, we find that 29.5 per cent of the nitrogen in the soil organic matter is made soluble in dilute acid, of which 7.6 per cent, or a little more than a quarter, of the nitrogen thus made soluble, is in the form of ammonia. This form of nitrogen is derived from the amides, which become hydrolyzed by the acid treatment. An almost equal amount (average 29.6 per cent) was made further soluble by

treatment with cold 80 per cent sulfuric acid followed by boiling with a 5 per cent solution of this acid for 5 hours.

When the residual soil after the two acid treatments was boiled with a 20 per cent hydrochloric acid solution for 30 hours, another 18 per cent of the nitrogen was made soluble; the residual soil still contained, on an average, 16.3 per cent of the original nitrogen. Whether this form of nitrogen, which is so highly resistant to the action of concentrated acid, is also most resistant to the action of microörganisms still remains to be determined. It is known at least that this form of nitrogen is low in plant proteins; the lowest amount of this nitrogen was found in the Alpine soil, considerable amounts of the organic matter of which are still in the process of decomposition.

TABLE 6

Influence of acid concentration and time of action upon the solubility of the "protein" nitrogen of the soil

SOIL NO.	TOTAL NITROGEN IN 100 GM. OF DRY SOIL	NITROGEN MADE SOLUBLE BY BOILING WITH 2 PER CENT HCl FOR 5 HOURS				NITROGEN MADE SOLUBLE BY 80 PER CENT SULFURIC ACID IN COLD (2 HOURS) FOLLOWED BY BOILING IN 5 PER CENT H_2SO_4 FOR 5 HOURS		NITROGEN MADE SOLUBLE BY BOILING WITH 20 PER CENT HCl FOR 30 HOURS		"HUMIN" NITROGEN LEFT IN RESIDUE AFTER BOILING WITH 20 PER CENT HCl FOR 30 HOURS		TOTAL NITROGEN ACCOUNTED FOR
		Nitrogen in solution		Ammonia in solution								
	mgm.	*mgm.*	*per cent of total*	*mgm. N*	*per cent of total N*	*mgm.*	*per cent of total*	*mgm.*	*per cent of total*	*mgm.*	*per cent of total*	*per cent*
4	239	64.8	27.1	23.1	9.7	68.7	28.7	53.4	22.3	37.2	15.6	93.7
6	154	51.6	33.5	12.7	8.2	52.7	34.2	33.5	21.8	18.1	11.8	101.3
10	443	170.1	38.4	43.8	9.9	133.8	30.2	48.1	10.9	41.2	9.3	88.8
16	670	151.1	22.5	41.4	6.2	202.4	30.2	104.0	15.5	176.5	26.3	94.5
18	332	85.8	25.8	24.4	7.3	104.4	31.4	82.3	24.8	56.2	16.9	98.9
21	398	117.4	29.5	30.4	7.6	92.8	23.3	62.4	15.7	82.8	20.8	89.3
22	440	121.6	27.6	33.5	7.6	116.1	26.4	70.7	16.1	84.5	19.2	89.3
29	315	100.0	31.7	26.9	8.5	102.0	32.4	51.9	16.5	33.4	10.6	91.2

In only one instance among the various soils analyzed, was there more than one horizon used, namely, in the case of the Brandon soil, with A and B horizons (nos. 21 and 22). The lower horizon was richer in organic matter than the upper horizon, as is shown by the higher carbon and nitrogen content, but the nature of the organic matter was nearly identical in both instances. This can be readily seen from the almost exact carbon-nitrogen ratio, and from the nitrogen distribution, as well as from the relative abundance of the non-nitrogenous complexes. The reaction of both horizons was also nearly the same. These results, as well, point to the possible applications of the method to the study of the nature of soil organic matter.

The analyses of two other horizons of one soil taken from different profiles of a solonietz type of soil near Fargo, North Dakota, are recorded in table 7. In the analyses of these two soils, the cold- and hot-water-soluble portions of the organic matter were also included. The results agree fully with the proximate analyses of the other soils and show again only minor differences in the chemical

composition of the two horizons, which, however, may prove important when more information has accumulated.

Two theories are current at present in the literature in regard to the chemical nature of the soil organic matter: According to one theory, the soil "humus" is made up of a few simple, well-defined chemical compounds, the so-called "humic acids" and "humins," which are believed to be produced from plant and animal residues added to the soil by certain simple chemical or biological processes. According to the other theory (14), the soil organic matter is made up of a large number of chemical constituents. Numerous organic compounds can be isolated from the soil, provided the proper methods are available.

The proponents of the first theory sought to clarify our conception of the nature of soil organic matter by determining the chemical and physico-chemical properties of two or three of the "amino acids." In most cases, however, these

TABLE 7

The chemical nature of the organic matter of two horizons of a Chernozem profile, at Fargo, N. D.

ORGANIC COMPLEXES	HORIZON A	HORIZON B
Organic matter by ignition.....*per cent*	12.63	12.95
Total carbon.....*per cent*	4.80	5.09
C x 1.72.....	8.26	8.75
Total nitrogen.....*per cent*	0.400	0.379
C/N.....	12.0	13.0
Proximate chemical composition, on per cent basis of total organic matter (C × 1.72)		
Ether-soluble.....	2.72	2.18
Cold-water-soluble.....	1.16	1.01
Hot-water-soluble.....	1.31	1.24
Alcohol-soluble.....	0.98	0.91
Hemicelluloses.....	1.81	2.75
Cellulose	6.12	4.86
Lignin-humus complex.....	47.70	45.82
"Proteins" or organic nitrogenous compounds.....	30.27	27.08

acids were nothing but labels for compounds obtained by different methods of preparation rather than of well-defined chemical properties. The proponents of the second theory did not attempt to present a clear picture of the soil organic matter as a whole, if that is at all possible.

The results presented here demonstrate beyond any doubt that a detailed study of the chemical nature of soil organic matter will reveal the fact that it is very complex in composition. The complexity in chemical composition of plant materials and animal bodies has not prevented the plant chemist and animal biochemist from undertaking a detailed study of them, with the result that a great body of information on these two subjects has accumulated; the complexity of the soil organic matter as well need not prevent the chemist, preferably the organic chemist, interested in soil problems from investigating the nature of this organic matter.

In the study of soil chemistry, the greatest emphasis has been laid upon the inorganic constituents while the organic chemical problems have received but scant attention. Our knowledge of the organic chemistry of the soil, especially of that highly important group of soil constituents, the organic matter complex in nature and formed as a result of numerous microbiological processes of decomposition and of synthesis, is still a science to be developed. For the present, however, a simplified method based upon the more detailed analysis may be suggested here, which will enable the student of soils to obtain at least a certain insight into the nature of the organic matter in the various soils and serve as a beginning for further investigation.

SIMPLIFIED METHOD OF ANALYSIS OF SOIL ORGANIC MATTER

The methods previously utilized for the study of the chemical nature of soil organic matter are somewhat too complicated for routine analysis. An attempt has been made, however, to simplify the method of analysis. The following is a brief outline of such a simplified method:

The soil to be analyzed is air-dried, ground, and sieved. The air-dry material may be used for analysis but the results should be calculated on an oven-dry basis. Total nitrogen and total carbon determinations are made on the air-dry material. The results of the analysis of the organic matter should be calculated on the per cent basis of the total organic matter of the soil. This is obtained by multiplying the organic carbon content of the soil by 1.72. This factor will probably be modified when considerable information has accumulated concerning the chemical composition of the soil organic matter. For the present, however, this factor is as satisfactory as any other one (2.0 for example) that might be suggested.

Two 200-gm. quantities of the oven-dry soil are extracted with ether in Soxhlets for 10 to 16 hours. This is followed by hot alcohol extraction. The extraction with a mixture (1:1) of benzol-alcohol may be employed, following the ether extraction, or may be used in place of both ether and alcohol extractions. The ether extract is evaporated to a small volume, then transferred to a weighing bottle; the residue carefully dried and the bottle weighed. The alcoholic extract is evaporated in weighed dishes. The amount of material (fats, waxes, and resins) soluble in ether and in alcohol is thus obtained. The extraction with benzol-alcohol mixture, in place of the ether and alcohol extractions, is carried out in a similar manner. The material extracted from soil by benzol-alcohol was found to be nearly equivalent to that extracted by ether and by alcohol.

Fifty-gram quantities of the dried residual material are placed in beakers and treated with 25 cc. of 80 per cent sulfuric acid solution for 2½ hours in the cold. Next, 375-cc. portions of distilled water are added to the beakers and the diluted acid extract is heated for 5 hours in flowing steam. The digest is filtered through dried and weighed papers and the residue is washed thoroughly with water. The solution is made up to volume and the amount of reducing sugar (also total and ammonia nitrogen if desired) determined. The sugar serves as a measure of the total carbohydrate content of the soil organic matter.

The residue from the acid extraction is dried and weighed. Two 5-gm. quantities from each residue are used for total carbon determinations, and two 10-gm. quantities are used for total nitrogen determinations. The "lignin" or "lignin-humus" complex is then calculated from the carbon and nitrogen content of this residue.

A lowmoor peat and two Sassafras soils, one manured for a number of years and one unmanured, were used for this analysis. The results are given in

table 8. These results show that by this method of analysis one can account for 95 per cent of the soil organic matter in the form of four definite, more or less well-defined chemical groups.

This method has a number of distinct advantages over the alkali extraction methods frequently used formerly:

It enables us to determine at a glance the stage of decomposition of the organic matter in the soil. Fresh, slightly decomposed organic matter of plant origin will have a high carbohydrate fraction and low "protein" and "lignin-humus" fractions. The more advanced the stage of decomposition of the organic matter, the less will be the carbohydrate content and the larger will become the last two fractions.

The method enables us to divide the organic matter of the soil into groups that are convenient for further study. Each fraction can then be studied by itself.

The results brought out by this method show how fallacious it is to speak of "humified" and "non-humified" portions of the soil organic matter. One may speak, however, of the

TABLE 8

Proximate composition of soil organic matter

On the basis of organic matter content of soil*

NATURE OF SOIL	ETHER- AND ALCOHOL-SOLUBLE FRACTION	CARBOHYDRATE FRACTION, AS SUGAR	"PROTEIN"	"LIGNIN-HUMUS"	SUM OF ORGANIC MATTER ACCOUNTED FOR
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Lowmoor† peat 51	2.51	10.29	25.97	54.48	93.25
Manured soil,‡ 5A	2.75	12.78	30.83	43.36	89.72
Unmanured soil, 7A	2.88	10.58	34.57	42.32	90.35

* In the case of the mineral soils, the organic matter content is calculated by multiplying the organic carbon by the factor 1.724.

† The peat analysis is also calculated on an ash-free basis; the ash content of the peat was 10 per cent.

‡ Total carbon content of the manured soil is 1.75 per cent; of the unmanured, 0.82 per cent.

degree of decomposition as indicated by the chemical changes that have taken place in the distribution of the various constituents in plant residues after they have become incorporated in the soil and become a part of its organic matter.

SUMMARY

The most reliable method available at present for determining quantitatively the soil organic matter is based upon the determination of organic carbon, which is multiplied by 1.724 to give total soil organic matter.

The methods proposed at different times for measuring the abundance of "humus" or "humic acid" in the soil, based upon its extraction with alkalies, cannot be used for determining quantitatively the soil organic matter or even a definite fraction of it. In these methods the extraction of the soil with alkalies is either followed by the evaporation of the alkali, by the precipitation of the

so-called "humus" with an acid, or by the determination of the color of the solution extracted, which is then compared with a standard solution.

Those methods which are based upon the use of oxidizing agents, such as permanganate solution or 6 per cent hydrogen peroxide solution for partial oxidation of soil organic matter, cannot be used for determining its degree of decomposition. These reagents usually act upon different substances, partly of plant origin and partly synthesized by the microörganisms, which have no direct bearing upon the degree of decomposition of the plant organic matter.

A proximate method of analysis of soil organic matter is suggested, whereby 90 to 95 per cent of all the constituents of the organic matter of the soil can be accounted for in groups of definite chemical complexes.

It has been found, by this method of analysis, that the soil organic matter is made up largely of two groups of complexes: the lignin-like complexes, which can be referred to as "humus-lignin" or "soil-lignin;" and the nitrogenous complexes or "soil proteins." In addition to these two groups, the soil organic matter contains some fats, waxes, and resinous substances (ether- and alcohol-soluble substances), and certain carbohydrates, including various hemicelluloses and small amounts of cellulose-like substances.

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AVAILABILITY OF MANGANESE AND OF IRON AS AFFECTED BY APPLICATIONS OF CALCIUM AND MAGNESIUM CARBONATES TO THE SOIL[1]

H. B. MANN[2]

North Carolina Agricultural Experiment Station

Received for publication January 6, 1930

One of the first great contributions to plant production was the establishment of the fact that certain elements are essential to plant growth, which culminated in Liebig's complete list, from which he erroneously omitted iron. With this change, the original 10 essential elements have remained intact for about three-quarters of a century, although many investigators have produced evidence favorable to the inclusion of others.

The importance of manganese has been stressed, but until recently it has received little consideration. Within the past few years the coincidence between response to manganese fertilization and the alkalinity of certain naturally calcareous and limed soils has indicated a probable relation between soil reaction and the availability of manganese similar to that which has been apparently established for iron.

HISTORICAL REVIEW

Manganese

The distribution of manganese in nature has been extensively studied. Richard (40), Bertrand and Rosenblatt (2), and McHargue (25) have determined its distribution and localization in plants, and Robinson (41) has recently reported the distribution of manganese in soils and shown that the amount varies throughout a wide range. High concentrations of manganese in the soil have been observed to be toxic to plant growth by Ewell (7), Lindsey (23), Kelly (21), and Guthrie and Cohen (15). On the other hand, Skinner and Reid (46), McHargue (26), McLean and Gilbert (29), Schreiner (44), Skinner (45, 38), and Willis (51) have suggested a deficiency of this element in certain soils.

Little is known of the rôle of manganese in plant nutrition but many theories have been advanced to account for its effect on plant growth. A stimulating

[1] Taken from a thesis presented to the graduate faculty of Cornell University in partial fulfillment of the requirements for the degree of doctor of philosophy. Published with the approval of the director of the North Carolina Experiment Station as paper 36 of the Journal Series.

[2] The author wishes to express appreciation to Prof. J. A. Bizzell, under whose direction this investigation was conducted.

effect of small quantities has been reported by Nagaoka (34), Labergerie (22), Bertrand (1), and Molinari and Ligot (33); whereas Grandeau (14) and Carlier and Claussen (4) obtained no appreciable benefit from its use. Manganese has been associated with the formation of nitrogenous compounds in plants by Salomone (43), Olaru (36), Roscosolano (42), and Pietruszczynski (37). Skinner et al. (47), (46), from early investigations, were led to conclude that crop response from manganese was related to its effect on the oxidative processes of the soil. Later work by Deatrich (6) seemed to substantiate this. That manganese acts both as a fertilizing agent and a catalytic body was suggested by Boucher (3). However, the general acceptance of manganese as necessary for plant economy began with the work of McHargue (24) and the evidence appears conclusive with the observation by McLean (28) that a manganese solution forced into the leaves of chlorotic plants through the stomata was locally as effective as was the application of the manganese to the soil.

The works of Somay (48), Masoni (30), Neotin (35), Ricci and Barbera (39), and McHargue (26) indicate that any kind of acid increases the solubility or availability of manganese, although only small amounts of the total manganese in soils are soluble in water.

Iron

The conditions governing the availability of iron have been extensively reported by Gile and Carrero (12), Jones and Shive (20), Mazé (31), and Carr and Brewer (5), but, as shown by the work of Willis and Carrero (52), the solubility of iron and the amount available to plants cannot be taken as a certain determinant of the efficiency of iron. The hypothesis advanced by Hopkins and Wann (17) that iron is active only in the ionized form offers a very good explanation for this.

Manganese and iron relations

Masoni (30) found the solubility of manganese and iron similar on both acid and limed soils. Johnson (18), on the other hand, offers good evidence that inorganic iron compounds may be held so completely oxidized in the soil in the presence of native manganese as to be practically insoluble. Fulsutsome (8) obtained very little response from either iron or manganese when used singly, but obtained marked effect upon growth from joint applications.

A deficiency of any one of several elements will result in the failure of plants to develop a normal green color (32). Such an iron chlorosis has been reported by Gile (10, 11, 13) and by Johnson (18, 19); a chlorosis on heavily limed soils apparently associated with manganese deficiency has been observed by Hartwell (16), McLean and Gilbert (29), Gilbert, McLean, and Hardin (9), and Zimmerley (53).

The conflicting results obtained from the use of manganese and iron indicate that a response from these elements when applied to the soil can only be

expected under certain conditions, and that a chlorosis of plants may be due to a deficiency of either. In some of the tropical soils iron seems to be deficient, but in the Atlantic Coastal Plain the chlorotic condition of certain plants grown on the naturally calcareous soils and those receiving large applications of calcitic or dolomitic lime is apparently due to a manganese deficiency. This investigation was therefore undertaken to determine the availability of manganese and of iron as affected by applications of calcium and magnesium carbonates to the soil.

SOLUBILITY OF MANGANESE AND OF IRON IN DUNKIRK AND DUNBAR SOILS

Carr and Brewer (5) have shown that the solubility of manganese and iron in solution decreases as the H-ion concentration of the solution is decreased. It cannot be assumed, however, that the solubility in soils will be the same as in solutions. This experiment was made to determine the solubility of these elements in soils as affected by applications of calcium and magnesium carbonates.

Water-soluble iron and manganese in Dunkirk gravelly sandy loam

On February 23, 1928, air-dry Dunkirk gravelly sandy loam soil was pulverized and all rocks and roots were removed. Four kilograms of this soil was then thoroughly mixed and put into 1-gallon glazed pots. Duplicate pots were treated with equivalent amounts of calcium and magnesium carbonates varying in increments of 1,000 pounds from 0 to 10,000 pounds an acre.

The moisture content of this soil was maintained at 10 per cent of the air-dry weight with distilled water until June 9, when samples were taken representing each treatment, and H-ion concentration determinations were made by the quinhydrone method. A sample equivalent to 1,000 gm. of soil on an air-dry basis was also taken for the determination of water-soluble iron and manganese. Each of these samples was placed in an 8-liter glass bottle and agitated with 5,000 cc. of distilled water at regular intervals for 48 hours. The solution was then filtered through paper and 4,000 cc. of the filtrate evaporated to dryness over steam. The residue was twice taken up with concentrated nitric acid and evaporated to dryness. A 10-cc. aliquot of this soil extract concentrate was used for the determination of iron by the thiocyanate method as described in the Bureau of Soils Bulletin 31, 1926.

Twenty-five cubic centimeters of the soil extract concentrate was used for the determination of manganese by the periodate method of Willard and Greathouse (50) with minor modifications. Considerable difficulty was experienced in developing the permanganate color in the soil extract concentrates from soils receiving the heavier rates of application, especially of magnesium carbonate, on account of high concentrations of organic matter dissolved from the soils.

Increasing amounts of calcium and magnesium carbonates were attended by progressive decreases in the H-ion concentration although the two alkaline

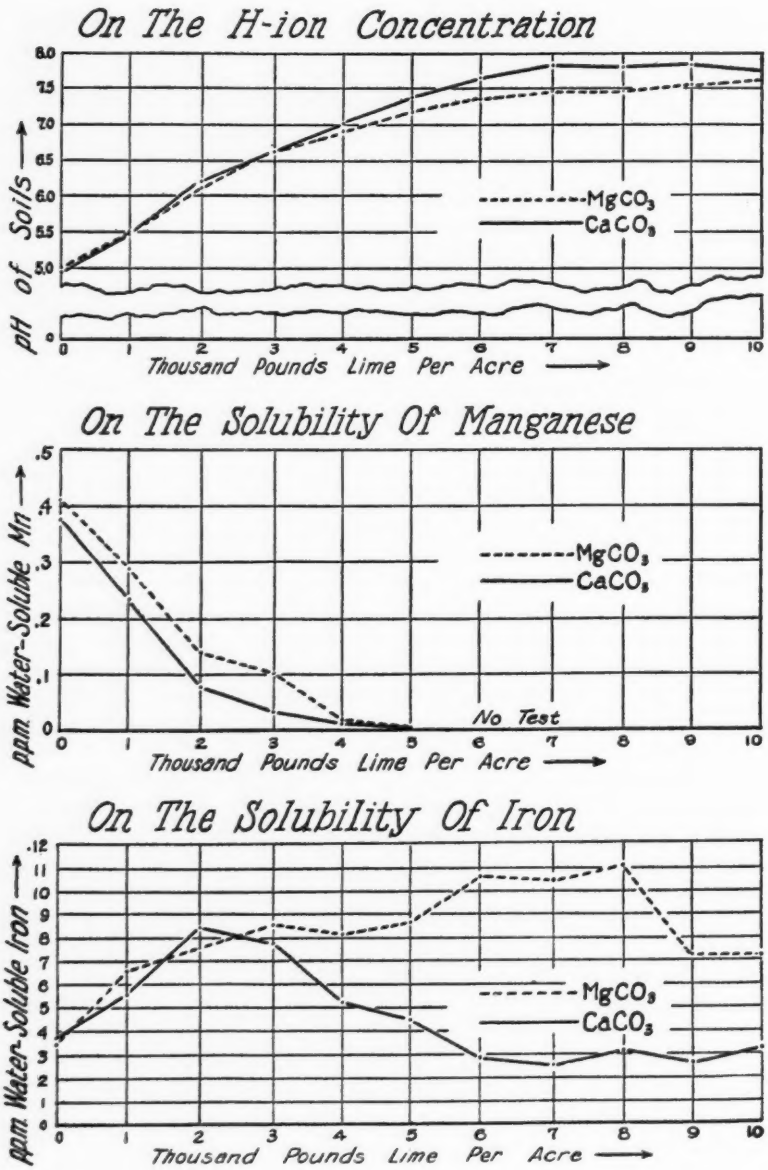

FIG. 1. EFFECT OF APPLICATIONS OF CALCIUM AND MAGNESIUM CARBONATES TO DUNKIRK GRAVELLY SANDY LOAM

agents were not equally effective in this respect, the differences being clearly shown by the H-ion concentration curves in figure 1. When the increments in pH are plotted as arithmetical rather than logarithmic values, the decrease in acidity with increase in calcium carbonate is approximately uniform, up to the 6,000-pound application, at a pH of 7.67 with minimum H-ion concentration of pH 7.8, reached with the 7,000-pound rate.

Increases in pH with increased magnesium carbonate were also uniform to about pH 7.19, which was reached with the rate equivalent to 5,000 pounds of calcium carbonate. Greater rates of application increased the pH up to the maximum at the 10,000-pound equivalent rate.

The effect of liming on the solubility of soil manganese was practically independent of the materials used. As shown by the manganese solubility curves in figure 1, there was a rapid and uniform decrease in solubility from the unlimed soil to the 2,000-pound rate of application, at a pH of about 6.2, with a gradual decrease to the 4,000-pound rate, at approximate neutrality. No manganese was found in extracts of the soil receiving more than 5,000 pounds of calcium carbonate or its equivalent in magnesium carbonate. Failure to show traces of manganese at the high rates was probably due to limitations of the analytical method.

In contrast to the regularity of the solubility curve for manganese is that for iron (figure 1). With the calcium carbonate treatments there is a sharp increase in solubility of iron up to a maximum concentration of 8.44 p.p.m. of soil at the 2,000-pound rate of application. Thereafter there is a gradual decrease in solubility to a virtual minimum at the 6,000-pound rate. With the magnesium carbonate treatments there is an increase in solubility to a maximum of 11.13 p.p.m., at the 8,000-pound equivalent rate, followed by a sharp decrease at the higher rates.

Obviously there is no simple relation between solubility of iron and reaction, or rate of liming, as was the case with manganese, nor is there a similarity between the effects of calcium and magnesium carbonates.

The shape of the solubility curve suggests the influence of two factors having opposite effects on the solubility of iron. One of these is probably the reaction tendency of the soil toward precipitation at the lower H-ion concentration, whereas the other may well be the increased solubility of iron in combination with the organic matter observed in the soil extracts at the higher rates of liming. The observation that greater amounts of organic matter appeared to be dissolved by the magnesium carbonate treatment would explain the divergence between the solubility curves with the calcium and the magnesium carbonates.

Water-soluble iron and manganese in Dunbar fine sandy loam

The soil used for this investigation was taken from an unfertilized and unlimed plat in a fertilizer experiment which had been conducted on the Pender County Branch Station Farm for 15 years. The soybeans grown in a

3-year rotation showed, on the limed end of the plat, a characteristic chlorotic condition of the upper leaves whereas those on the unlimed end were of normal green color.

On October 10, 1928, 4,000 gm. of this soil in air-dry condition was treated with equivalent amounts of calcium and magnesium carbonates varying from 0 to 10,000 pounds an acre and put into clean 1-gallon glazed pots. These potted soils were brought up to 10 per cent moisture with distilled water and maintained at this moisture content for approximately three months.

On January 15, 1929, samples were taken from each pot for the determination of water-soluble iron, manganese, and H-ion concentration. The H-ion concentration was determined by the quinhydrone electrode method, as in the case of the Dunkirk soil, and the soil extract for the determination of water-soluble iron and manganese obtained and concentrated as before.

For the determination of iron the colorimetric method of Stokes and Cain (49) was used, as it gave more consistent results than the method used with the Dunkirk soil. Colors were compared in a Bosch and Lomb colorimeter with standards of nearly equal concentration. For the determination of water-soluble manganese it was found that digestion with a small amount of sulfuric acid and potassium chlorate destroyed all traces of organic matter, permitting a more satisfactory development of color in the extracts of the heavily limed soils. Otherwise the method of analysis was essentially the same as with the Dunkirk soil. Treatments and analytical data are presented graphically in figure 2.

As with the Dunkirk soil, increases in the rate of liming progressively decreased the H-ion concentration, and the rate of decrease was uniform for both calcium and magnesium carbonates up to the 1,500-pound rate. From this point the rate of increase, as shown by the H-ion concentration curves in figure 2, was less rapid with magnesium carbonate, and no maximum was reached with the latter material at the heaviest rate of application, giving a pH of 7.4.

Increased rates of application of calcium carbonate uniformly increased the pH values up to the 5,000-pound rate and an approximate minimum H-ion concentration was reached at the 10,000-pound rate, with a pH of 7.53.

With allowances for the greater initial acidity of the Dunbar soil there is a striking similarity in the reaction curves of the two soils as regards both the calcium and magnesium carbonates. Calcium carbonate equivalent to 3,500 pounds an acre was required to raise the pH of the Dunbar soil from 5 to 7, whereas 4,000 pounds was required by the Dunkirk soil, indicating a slightly greater buffer value for the latter. Manganese was distinctly more soluble in the Dunbar than in the Dunkirk soil, both unlimed, as would be expected on account of the greater acidity of the Dunbar soil. The decrease in the solubility with increased rates of liming was much the sharper with the Dunbar soil. Probably on account of improved technique, determinable amounts of manganese were found in the Dunbar soil even at the heaviest rate of liming.

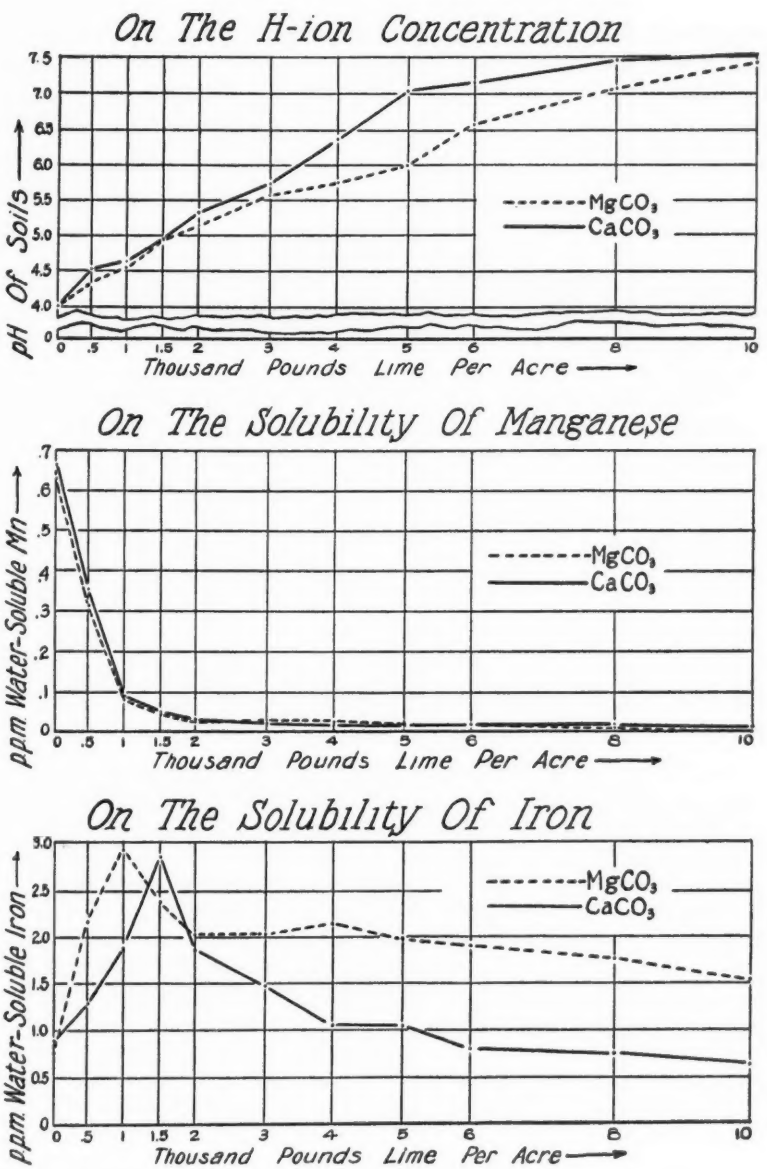

FIG. 2. EFFECT OF APPLICATIONS OF CALCIUM AND MAGNESIUM CARBONATES TO DUNBAR FINE SANDY LOAM

The effects of calcium and magnesium carbonates on the solubility of manganese were identical, as is shown in figure 2.

There was a very sharp increase in the solubility of iron resulting from the addition of lime to the acid soil. As shown in figure 2, by the solubility curves for iron, this reached a maximum with calcium carbonate at the 1,500-pound rate, beyond which there was a decrease in solubility. At the 6,000-pound rate the solubility of iron was the same as that of the unlimed soil and the minimum was reached at the 10,000-pound rate of liming.

With magnesium carbonate the maximum solubility was found with the 1,000-pound equivalent rate of application, with a gradual decrease in solubility from this point with increasing rates of application. The minimum solubility of iron with magnesium carbonate did not, however, become as low as that of unlimed soil.

The solubility curves for iron in the Dunkirk and Dunbar soils have some striking similarities. There is a sharp increase in solubility following the application of lime with maxima reached at less than the maximum pH value. The increase in solubility was better sustained with the greater amounts of magnesium carbonate than with calcium carbonate. This hitherto unnoted behavior of iron as affected by liming is apparently common to soils of widely differing characteristics and involves a principle not evident from the data at hand, although the influence of liming on the solubility of the soil organic matter seems to be a pertinent observation.

EFFECT OF CALCIUM AND MAGNESIUM CARBONATES ON GROWTH AND COMPOSITION OF SOYBEANS

The use of large amounts of dolomitic limestone for tobacco and of both calcitic and dolomitic limestone for peanuts and other crops on some of the dark, sandy, poorly drained soils of the Atlantic Coastal Plain has frequently produced a chlorotic condition of succeeding soybean and other crops. Hence this experiment was made to determine the effect of varying applications of calcium and magnesium carbonates on growth and on the manganese and iron content of soybeans.

On October 10, 1928, gallon glazed pots were set up in triplicate with Dunbar fine sandy loam, as used for solubility determinations, and maintained at 10 per cent moisture until January 8, 1929, when 11 soybean seeds of uniform size and color were planted in each pot. The moisture content of the soil was raised to 20 per cent of the air-dry weight and maintained at this content throughout the growing period.

Notes taken on January 15 indicated no injury to germination from applications of calcium carbonate even at the maximum rate. A delayed germination was noted, however, on the pots receiving high applications of magnesium carbonate. Observations 3 days later showed no injury to seedlings from the use of calcium carbonate, but applications of magnesium carbonate above 4,000 pounds greatly reduced the vitality of the seedlings, those growing in pots receiving the highest amounts dying before the first leaves developed.

On January 20, the beans were thinned to 8 plants in each pot. When these were about 4 weeks old a chlorosis appeared on the lower leaves of all plants receiving applications of calcium carbonate greater than 5,000 pounds an acre, the severity of the chlorosis increasing with the higher rates of application. This symptom, being more pronounced on the lower leaves, did not resemble manganese deficiency chlorosis but may have been due to a magnesium deficiency resulting from the excessive applications of lime. Plate 1, figure 1 illustrates a typical case of this condition.

The decrease in vitality from heavy applications of magnesium carbonate observed when the beans were in the seedling stage increased with the age of the beans, those growing in pots representing treatments 18, 19, and 20 finally dying. As the young leaves developed on these plants they turned brown around the edge, dried, and dropped off. This injury was slight on treatment 15, but increased with magnesium applications.

Because this soil had no fertilization in the field for 15 years, the plants in the pots made limited growth. Therefore when the pods were about half grown and no chlorosis had developed that could be definitely distinguished as due to a deficiency of manganese, two of the series were cut and the roots removed from the soil. One series was fertilized with a solution of one-half gram of ammonium phosphate and one-half gram of potassium phosphate to each pot. The second series was not fertilized. These pots were replanted with soybeans as has been reported previously.

The third series, which was allowed to grow to maturity, gave no indication of a manganese or iron deficiency. The plants of the replanted pots reacted to the calcium and magnesium carbonate treatments in the early stages of growth as did those of the first crop, but the fertilized series made a more vigorous growth than did the unfertilized. When the plants were about half grown, those receiving applications of calcium carbonate above 6,000 pounds on both fertilized and unfertilized series began to show a chlorotic condition of the upper leaves indicative of a manganese deficiency. This diagnosis was confirmed when a green color developed a few days after the chlorotic leaflets were dipped into a 1–1000 solution of manganese sulfate. The magnesium carbonate treatments did not produce a distinct case of chlorosis, probably because the heavy applications were very injurious, no soybeans surviving on treatments 18, 19, and 20. The increased chlorotic condition with increasing calcium carbonate applications is shown in figure 2 of plate 1. Plate 1, figure 3 shows the effect of the magnesium carbonate treatments at the same age.

On June 17, after the pods had formed, the soybeans were cut and sacked separately with the leaves which had fallen from the respective plants. The entire crop from each pot constituted the sample for analysis. The oven-dry weight was obtained by drying for 72 hours at 100°C. The samples were then thoroughly charred at low red heat, moistened with 5 cc. 1–1 nitric acid and evaporated nearly to dryness over a steam bath. The char was then taken up with 1–9 hot nitric acid, transferred to a filter, and washed with 100 cc. of the

hot nitric acid. The residue and filter paper were burned to a white ash and mixed with the filtrate, which was evaporated nearly to dryness before being made up to 100 cc. with 1–9 nitric acid. For the determination of manganese, 25-cc. aliquots were taken, and for iron 10-cc. aliquots. As in previous analytical work, the Stokes and Cain (49) method was used for the determination of iron, and the Willard and Greathouse (50) method for manganese. In table 1 are presented the results obtained for the fertilized series.

TABLE 1

Results with soybeans fertilized and grown on soils treated with $CaCO_3$ and $MgCO_3$

POT NUMBER	$CaCO_3$	DRY WEIGHT OF PLANT	MANGANESE CONTENT OF PLANTS			IRON CONTENT OF PLANTS			Mn REMOVED PER POT	Fe REMOVED PER POT
			First determination	Second determination	Average	First determination	Second determination	Average		
		gm.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*mgm.*	*mgm.*
Check	0	7.34	109.0	99.1	104.1	247.6	236.8	242.2	0.764	1.78
1	500	10.83	76.9	73.8	75.4	196.4	205.1	200.8	0.817	2.18
2	1,000	8.41	63.4	56.0	59.7	205.0	230.9	218.0	0.502	1.83
3	1,500	10.26	39.8	41.0	40.4	170.9	173.9	172.4	0.415	1.77
4	2,000	10.01	33.0	29.6	31.3	195.9	185.2	190.6	0.313	1.91
5	3,000	13.47	26.5	25.2	25.9	157.9	138.7	148.3	0.349	2.00
6	4,000	10.64	23.5	24.0	23.8	195.8	208.9	202.4	0.253	2.15
7	5,000	8.61	22.3	20.6	21.5	232.2	207.3	219.8	0.185	1.89
8	6,000	7.31	19.0	18.7	18.9	258.1	223.9	241.0	0.138	1.76
9	8,000	9.07	12.4	11.6	12.0	245.1	232.2	238.7	0.109	2.17
10	10,000	7.51	11.3	8.3	9.8	256.2	242.2	249.2	0.074	1.87
	$MgCO_3$									
Check	0	3.78	124.5	117.6	121.1	224.2	216.0	220.1	0.458	0.83
11	420	7.37	79.8	67.9	73.9	226.2	212.0	219.1	0.545	1.63
12	840	9.91	59.4	50.5	55.0	213.0	200.2	206.6	0.545	2.05
13	1,260	7.16	48.6	43.0	45.8	249.5	232.8	241.2	0.328	1.73
14	1,680	7.79	36.7	36.7	36.7	256.8	256.8	256.8	0.286	2.00
15	2,520	9.12	31.3	31.3	31.3	210.8	193.9	202.4	0.286	1.85
16	3,360	6.65	25.9	21.6	23.8	254.9	246.5	250.7	0.158	1.67
17	4,200	2.93	24.2	20.0	22.1	232.2	213.3	222.8	0.065	0.65

Pots 18, 19, and 20, receiving 5,040 pounds, 6,720 pounds, and 8, 400 pounds of $MgCO_3$, respectively, produced no growth.

Liming at moderate rates with both calcium and magnesium carbonates increased the yield. More than the 3,000-pound rate resulted in a slight decrease with the calcium carbonate and distinct evidences of toxicity with the magnesium carbonate. The amount of manganese in the plants decreased very rapidly with increased applications of calcium and magnesium carbonates to the soil. An examination of the data shows that the manganese content of the plants follows a curve very similar to the solubility curve for the soil.

It seems from these results that the manganese content of plants might,

under certain conditions, be as accurate an index of the available manganese in the soil as is the water-soluble manganese in the soil extract. The total amount of manganese removed by each pot decreased as the applications of calcium and magnesium were increased, except in the case of check no. 2, on which the beans made only a very slight growth.

The iron content of the soybeans varied little with the different liming treatments, and no correlation was evident between the iron content of plants

TABLE 2

Results with soybeans unfertilized and grown on soils treated with $CaCO_3$ and $MgCO_3$

POT NUMBER	$CaCO_3$	DRY WEIGHT OF PLANTS	MANGANESE CONTENT OF PLANTS			IRON CONTENT OF PLANTS			Mn REMOVED PER POT	Fe REMOVED PER POT
			First determination	Second determination	Average	First determination	Second determination	Average		
		gm.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*mgm.*	*mgm.*
Check	0	4.77	118.1	125.1	121.6	230.3	262.0	246.2	0.580	1.17
1	500	5.89	77.2	81.8	79.5	212.3	226.4	219.4	0.468	1.29
2	1,000	5.51	51.8	58.1	55.0	183.3	193.1	188.2	0.303	1.04
3	1,500	4.47	42.6	46.4	44.5	188.0	203.4	195.7	0.199	0.88
4	2,000	4.63	29.8	31.6	30.7	212.8	216.0	214.4	0.142	0.99
5	3,000	4.81	23.5	23.1	23.3	232.2	225.9	229.1	0.112	1.10
6	4,000	5.68	17.1	17.4	17.3	222.9	227.2	225.1	0.098	1.28
7	5,000	5.78	13.3	14.1	13.7	206.0	226.1	216.1	0.079	1.25
8	6,000	5.11	12.9	13.7	13.3	217.4	217.4	217.4	0.068	1.11
9	8,000	5.31	11.2	12.6	11.9	206.9	221.5	214.2	0.063	1.14
10	10,000	5.26	10.0	9.8	9.9	195.0	218.3	206.7	0.052	1.09
	$MgCO_3$									
Check	0	2.24	102.0	106.3	104.2	251.5	223.2	237.4	0.233	0.53
11	420	4.48	66.1	68.7	67.4	255.1	279.0	267.1	0.302	1.20
12	840	4.86	50.8	45.7	48.3	207.9	283.9	245.9	0.235	1.20
13	1,260	5.45	34.1	31.5	32.8	188.2	209.7	199.0	0.179	1.09
14	1,680	4.30	21.8	21.9	21.9	255.6	300.1	277.9	0.094	1.20
15	2,520	5.01	17.3	19.0	18.2	257.5	279.2	268.4	0.091	1.35
16	3,360	5.30	12.1	13.8	13.0	235.9	210.8	223.4	0.069	1.18
17	4,200	0.68	0	0	0	262.6	245.1	253.9	0	0.17

Pots 18, 19, and 20, receiving 5,040 pounds, 6,720 pounds, and 8,400 pounds of $MgCO_3$, produced no growth.

and calcium or magnesium carbonate applications. The evidence for a manganese deficiency as a cause of the chlorosis, which was shown by the recovery when the chlorotic leaflets were dipped into a manganese solution, is supported by the analytical data, since the chlorotic plants contained as much iron as but less manganese than the green plants.

Results with soybeans grown on the unfertilized series are given in table 2. The yields were only about half as great as in the fertilized series, and no distinct increases were made as a result of liming. The manganese content

of the soybeans representing the various treatments was very similar in respect to the amount absorbed and the effect of liming to those obtained on the fertilized series. No correlation was evident between the iron content of the soybeans and calcium or magnesium carbonate applications. Again it appears that the chlorosis on these soils could not have been due to a deficiency of iron, since the chlorotic plants contained as high a percentage of iron as the green plants.

CAUSE AND CONTROL OF CHLOROSIS OF SOYBEANS ON LIMED SOILS

In connection with the study of the solubility of soil manganese and iron it seemed advisable to examine some of the evidences of response of plants to compounds of these two elements. Soybeans were chosen as test plants because of their known susceptibility to both iron- and manganese-deficiency chlorosis.

Four thousand grams of air-dry Coxville sandy loam soil was weighed in gallon glazed pots and planted with Mammoth Yellow soybean seed. This soil though normally acid had been heavily limed to a pH of 8.4 and had produced chlorotic soybeans in the field. The seedlings were thinned to eight plants and grown under uniform conditions, for three weeks, by which time the apical leaves of all plants were chlorotic. These soybeans were used for the following experiments.

Effect of fertilization on growth and composition of soybeans

Ten cubic centimeters of a one part per hundred solution each of $MnSO_4$, $FeSO_4$, and $MgSO_4$ was applied separately to duplicate pots of uniformly chlorotic soybeans. After seven days the soybeans fertilized with $MnSO_4$ were decidedly greener than those receiving the other treatments. The response to these treatments three weeks after the applications were made is shown in plate 2, figure 1. The plants receiving manganese had developed much larger and greener leaves than those in any of the other pots, whereas the pots receiving magnesia and iron were somewhat greener than the check, indicating a slight response from these materials.

Analyses of the chemicals used for this investigation showed that the manganese sulfate contained considerable iron and that the iron sulfate gave a distinct test for manganese, whereas the magnesium sulfate contained only very slight traces of either. For this reason it was decided to repeat the experiment using purer sources of iron, manganese, and magnesium and including several additional elements.

Using the same cultural methods as before, 10 cc. of a one part per thousand solution of each of manganese sulfate (iron-free), iron sulfate (manganese-free), iron citrate, iron tartrate, copper sulfate, barium chloride, and magnesium sulfate was applied to uniformly chlorotic pots of soybeans. Five days after the treatment the beans treated with manganese sulfate were distinctly greener

than any of the others, which showed no improvement in comparison with the controls. All plants were allowed to grow until pods began to form.

The beans from a check pot and those treated with iron chloride, iron citrate, and manganese sulfate were cut and the manganese and iron contents determined. The methods used for the determination of iron and manganese were the same as those used for the soybean plants, the Stokes and Cain (49) colorimetric method being used for iron determinations and the periodate method of Willard and Greathouse (50) for the determination of manganese. Minor modifications of both were made to adapt them to the materials analysed. Results are presented in table 3.

It will be noted from this table that the plants treated with manganese sulfate made nearly twice as much growth as any of the others. No significant increase in growth was evident from either of the iron treatments over the check. The plants treated with manganese had approximately twice the manganese content of either the check or iron-treated plants, and removed over three times as much from the soil, whereas the iron removed by each pot was

TABLE 3

Manganese and iron contained in soybeans fertilized with manganese and with iron

TREATMENT	DRY WEIGHT OF PLANTS	MANGANESE CONTENT OF PLANTS			IRON CONTENT OF PLANTS			Mn REMOVED PER POT	Fe REMOVED PER POT
		First determination	Second determination	Average	First determination	Second determination	Average		
	gm.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*mgm.*	*mgm.*
$FeCl_2$	12.28	9.0	7.2	8.1	310.0	325.6	317.8	0.100	3.90
FeCit	13.54	8.4	6.7	7.6	295.6	295.6	295.6	0.103	4.00
$MnSO_4$	22.69	14.6	14.5	14.6	196.0	157.0	176.5	0.331	4.01
Check	13.11	8.0	6.9	7.5	305.2	256.1	280.7	0.098	3.68

nearly the same for all treatments. The lower content of iron in the plants receiving manganese seems, therefore, to have been due to the increased growth resulting from the treatment, although there may have been some decrease in the amount of available iron as a result of the manganese addition.

Treatments on the foliage as a corrective of chlorosis

The increased growth and recovery from chlorosis of the plants receiving manganese would establish the fact of a deficiency of that element in the soil if it were not for the possibility of the manganese effect being the result of a secondary reaction in the soil. This possibility was investigated by spraying the foliage of chlorotic plants with 1–1,000 solutions of the same compounds as were added to the soil, precautions being taken in all cases to prevent the spray materials from dripping onto the soil. As shown in plate 2, figure 2, the manganese sulfate as a spray was as effective in overcoming the chlorosis as when applied to the soil and, similarly, no response was noted from any of the

other materials. The evidence appears conclusive that the chlorosis observed is specifically due to manganese deficiency.

A more striking demonstration of this method of showing nutrient deficiencies was made by dipping the center leaflets of chlorotic leaves into the solutions tested as sprays on the foliage. The response is illustrated in plate 3. This method permits of the testing of several elements on a few plants, with untreated leaflets of each leaf serving as controls.

The method is probably limited to those elements required by plants in almost infinitesimal amounts. The corrective effect of manganese solutions applied to the leaves is evident at dilutions as great as one part of manganese sulfate in 100,000 of water (Pl. 4, fig. 1). The recovery of green color by chlorotic leaves following the application of manganese to the foliage was prompt, a response having been noted within 24 hours.

Severely chlorotic plants are characterized as having the lower leaves persistently green while the apical leaves are small and yellow. The function of manganese applied to the leaves appears to be local rather than systemic, for single leaflets, dwarfed by the deficiency, were brought to normal color and size by dipping into a manganese solution (Pl. 4, fig. 2).

SUMMARY

Calcium and magnesium carbonates added to Dunkirk gravelly sandy loam in pots decreased acidity. Both carbonates affected the reaction equally at the lower rates of application, but the magnesium carbonate was less effective at the higher rate.

Liming decreased the solubility of soil manganese, the rate being rapid with the smaller applications. Manganese became relatively insoluble with applications above 5,000 pounds an acre.

The solubility of iron was increased by moderate liming but decreased at the higher rates. The increase was better sustained with magnesium carbonate than with calcium carbonate and the minimum solubility with the former was never less than that in the unlimed soil. The increase in solubility of iron consequent to liming may be due to combination of iron with the soluble organic constituents of the soil dissolved by lime.

With Dunbar fine sandy loam the response to liming was similar to that with Dunkirk soil. The Dunbar soil was the more acid and was less well buffered. Manganese was more soluble but more readily precipitated, although some manganese was soluble at the maximum rate of liming.

Solubility curves for iron had the same characteristics in both soils with maxima at low rates of liming and indicated a greater solubility with magnesium carbonate at the higher rates.

Soybeans grown in pots of the Dunbar soil with calcium or magnesium carbonate and with fertilization responded to moderate liming. Heavy applications of calcium carbonate decreased the yields from the maximum whereas the larger amounts of magnesium carbonate were toxic.

The manganese absorbed by the plants exhibited a curve closely following that of the solubility of manganese in the soil and showing the manganese to be largely precipitated, and absorption decreased at the point of maximum yield.

No significant differences were found in the effects of liming on iron absorption.

Unfertilized plants on this soil made less growth and gave no response to lime other than a change in the color of the foliage, and that due to the toxic effect of heavy applications of magnesium carbonate. The effect of liming on the absorption of manganese and iron was similar to that with the fertilized plants.

A chlorosis of soybeans that had been observed in the field on limed soils was reproduced in pots on a Coxville sandy loam limed to pH 8.4. This chlorosis, typified as being more severe at the apex of the plant, was remedied by applications of manganese sulfate either to the soil or to the chlorotic leaves as a spray. Very dilute solutions were effective when applied directly to the foliage. Iron, magnesium, copper, and barium salts applied to the soil or to the foliage, were without effect on the chlorosis. Diagnosis of manganese-deficiency chlorosis on soybeans is very effectively made by observing the reponse after one leaflet of a leaf was dipped into a manganese solution.

Manganese-deficiency chlorosis is not a systemic deficiency, as localized applications of manganese salts result in complete recovery of the part both in color and size.

CONCLUSION

The solubility of iron and manganese in soils, the iron and manganese contained in plants, and the response from applications of various salts to the soil and on the foliage of plants indicate that the chlorosis of soybeans grown on certain heavily limed soils is not associated with a deficiency of iron, but is specifically due to a deficiency of manganese.

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PLATE 1

EFFECTS OF CALCIUM AND MAGNESIUM CARBONATES ON SOYBEANS

FIG. 1. A chlorosis of soybeans, severe on the lower leaves, consequent to heavy applications of calcium carbonate.

FIG. 2. Effect of applications of calcium carbonate on growth and color of soybeans.

FIG. 3. Effect of applications of magnesium carbonate on growth and color of soybeans.

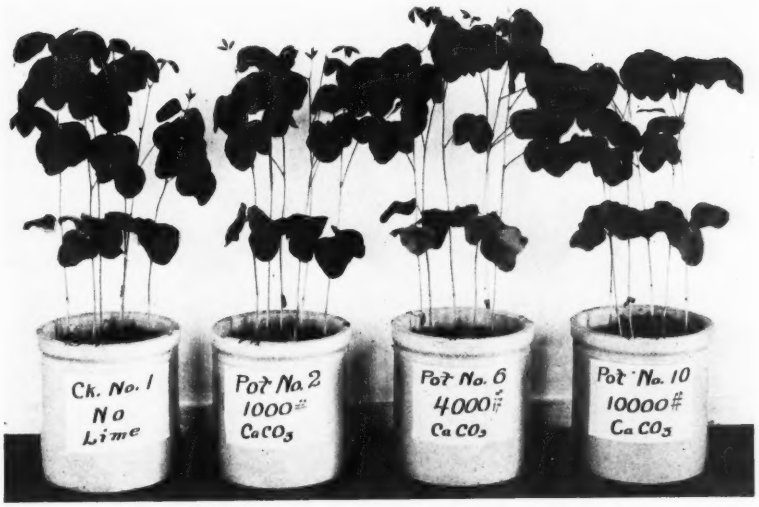

FIG. 1

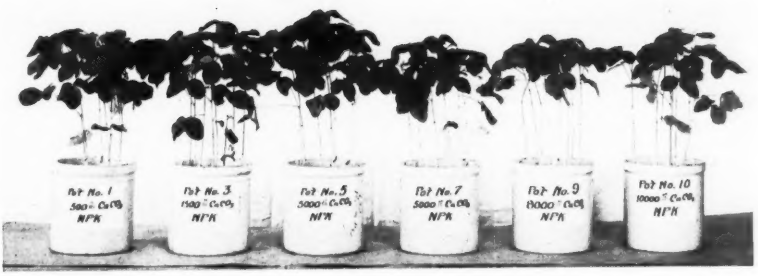

FIG. 2

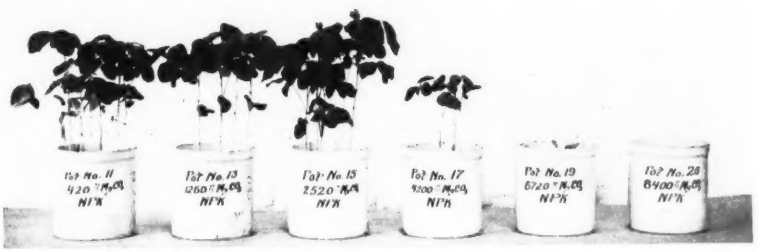

FIG. 3

PLATE 2

EFFECT OF FERTILIZERS AND SPRAYS ON CHLOROTIC SOYBEANS

FIG. 1. Chlorotic Soybeans fertilized with manganese sulfate, iron sulfate, and magnesium sulfate.

FIG. 2. Chlorotic soybeans sprayed with manganese and iron.

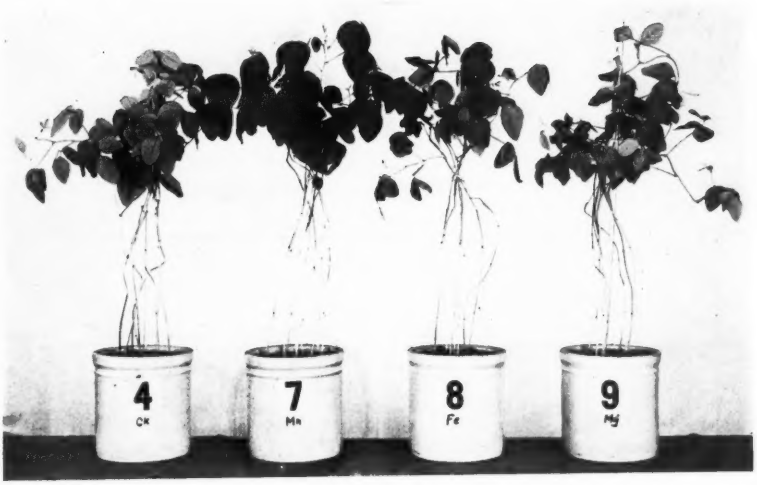

FIG. 1

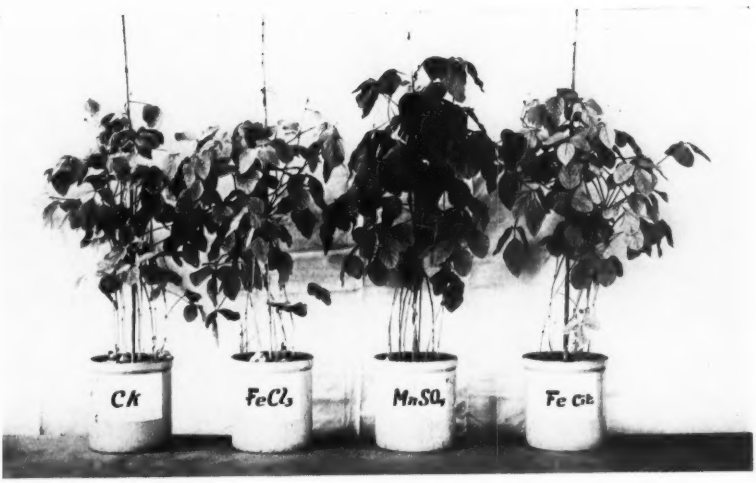

FIG. 2

PLATE 3

EFFECT OF DIPPING THE CENTER LEAFLET OF CHLOROTIC SOYBEAN LEAVES INTO SOLUTIONS AS INDICATED

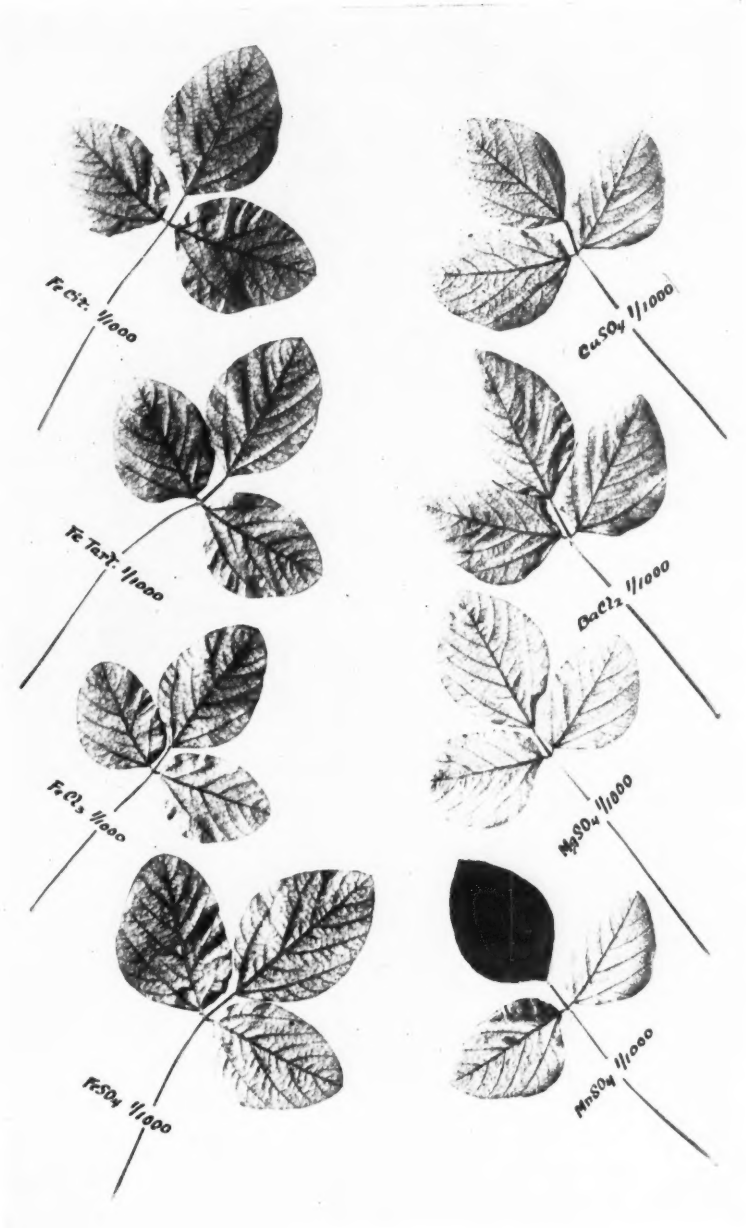
Fe Cit. 1/1000
Fe Tart. 1/1000
Fe Cl3 1/1000
FeSO4 1/1000
CuSO4 1/1000
BaCl2 1/1000
MgSO4 1/1000
MnSO4 1/1000

PLATE 4

EFFECT OF MANGANESE SULFATE ON CHLOROTIC SOYBEAN LEAVES

FIG. 1. Effect of dipping the center leaflet of chlorotic soybean leaves into different concentrations of manganese sulfate.

FIG. 2. Response of young chlorotic soybean leaflets to dipping into a 1–1,000 solution of manganese sulfate.

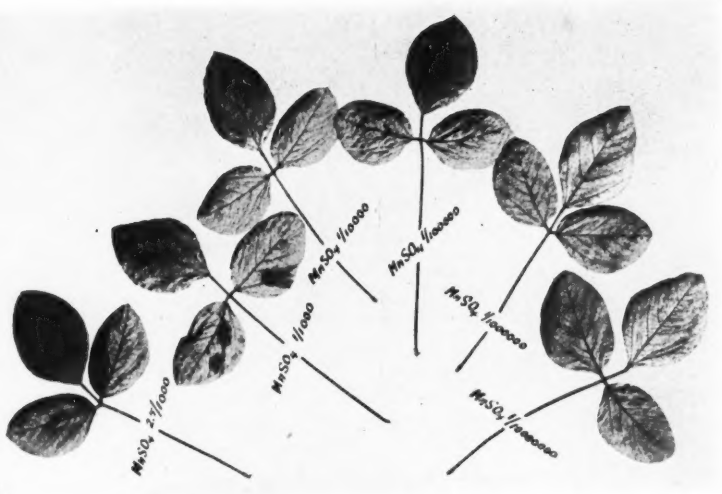
MnSO4 2%000
MnSO4 1%000
MnSO4 1%0000
MnSO4 1%00000
MnSO4 1%000000
MnSO4 1%0000000

FIG. 1

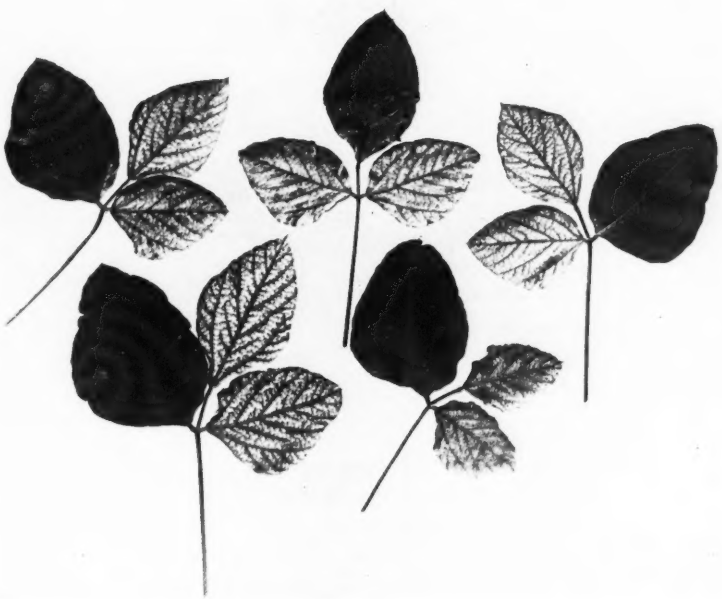

FIG. 2

COMPOSITION OF NATURAL ORGANIC MATERIALS AND THEIR DECOMPOSITION IN THE SOIL: V. DECOMPOSITION OF VARIOUS CHEMICAL CONSTITUENTS IN PLANT MATERIALS, UNDER ANAEROBIC CONDITIONS[1]

FLORENCE G. TENNEY AND SELMAN A. WAKSMAN

New Jersey Agricultural Experiment Station

Received for publication January 3, 1930

It has been shown in a previous contribution (6) that the environmental conditions, such as reaction, moisture supply, and aeration, play an important rôle in the decomposition of various complex organic materials of plant origin. These conditions are influential in modifying the nature of the microörganisms taking an active part in the decomposition processes as well as the amount and extent of the organic complex as a whole, and its individual chemical constituents in particular. If the decomposition takes place under aerobic conditions, with a sufficient supply of air and an optimum moisture and temperature, the reduction in the sugars, celluloses, hemicelluloses, fats, and proteins accounts for most of the plant materials that have disappeared. The lignins are much more resistant to decomposition than the polysaccharides and proteins and tend to accumulate; however, this resistance is relative rather than absolute in nature. When the plant material has a low nitrogen content, its decomposition is found to be accompanied by a relative and absolute increase in the protein or organic nitrogen content, due to the synthesizing activities of the microörganisms. These organisms derive their energy principally from the decomposition of the celluloses and hemicelluloses. As a result of this, the addition of inorganic nitrogen salts to plant materials poor in nitrogen and rich in polysaccharides will favor its breakdown by microörganisms.

Even after a considerable period of time (one to two years), a certain residue is left as a result of the decomposition of the plant materials under aerobic conditions at an optimum temperature and moisture. This residue has all the properties of the soil "humus." It is made up chiefly of lignins or modified lignin complexes, of proteins and other organic nitrogenous complexes largely of microbial origin, of hemicelluloses partly of plant and partly of microbial origin, and of various substances in the process of decomposition or resulting from the decomposition of the plant constituents. This "humus" is not in a state of equilibrium but undergoes constant change in chemical composition.

[1] Journal Series paper of the New Jersey Agricultural Experiment Station, department of soil chemistry and bacteriology.

A large part is soluble in alkali, giving a dark pigment, and is reprecipitated by acids, thus having the properties of the "humic acids" of the soil.

To demonstrate the possible differences that may result from the decomposition of organic substances under aerobic and anaerobic conditions, the following illustrations may be cited:

When celluloses are decomposed under aerobic conditions by bacteria, fungi, or actinomyces, they do not yield any organic acids or alcohols, but they result in a more or less complete destruction of the polysaccharide with the liberation of considerable quantities of carbon dioxide; from 20 to 30 per cent of the energy may be utilized for the synthesis of microbial cell substance, rich in organic nitrogenous complexes, such as proteins, and of hemicelluloses and other extra-cellular and intra-cellular products of microbial metabolism (2). Whether the slimy substances produced are synthesized hemicelluloses or intermediary oxycelluloses (11) still remains to be determined. However, when celluloses are decomposed by bacteria under anaerobic conditions, large quantities of organic acids are produced (3, 4, 7).

Under natural aerobic conditions, as in aerated fields or loosely kept manure piles, there is a continuous destruction of organic matter even where large quantities of plant residues are added to the soil every year. However, under anaerobic conditions, as in both acid and neutral peat bogs, there is a continuous accumulation of organic matter, the amount of decomposition being less than the yearly addition of the organic matter by natural vegetation.

Although certain initial processes of decomposition may well go on under anaerobic conditions, as in sewage tanks, for the complete disintegration of the organic residues, proper aeration is required.

In a study made on the decomposition of immature oak leaves under aerobic and anaerobic conditions (10), it was shown that when the leaf material was saturated with water, the celluloses and hemicelluloses were decomposed much more slowly than when the material was under aerobic conditions; the fats and waxes were much more resistant to decomposition and the lignins were preserved almost quantitatively, whereas the protein content of the anaerobic material was considerably greater than that of the aerobic compost because of the greater decomposition of the proteins and losses of the nitrogen in the form of ammonia in the aerobic compost.

These few examples are sufficient to illustrate the differences that may be expected from a comparative study of the decomposition of organic substances under aerobic and anaerobic conditions. They will probably tend to throw some light upon the differences in the nature of organic matter in aerated soil and in peat bogs, if such a difference exists at all.

In order to determine to what extent the decomposition processes are influenced by a change in environmental conditions, a series of experiments were outlined in which the material undergoing decomposition was saturated with water to exclude the free admission of oxygen. Under these conditions, the fungi, most actinomyces, and many aerobic bacteria are excluded, except at the very surface of the material, which is in contact with air. The anaerobic and facultative anaerobic bacteria are largely concerned in the decomposition of the various chemical plant constituents under these conditions. To bring about anaerobic conditions, it is not necessary to exclude the air completely. It is sufficient to cover the plant material undergoing decomposition completely with water, thus reducing the oxygen tension sufficiently to favor the develop-

ment of anaerobic organisms and make conditions unfavorable for the strict aerobic forms.

Five different plant materials, the same as those used in the aerobic decomposition study reported previously (6), were selected for this study; namely, (*a*) mature corn stalks and leaves, (*b*) rye straw, (*c*) mature yellow oak leaves, freshly fallen to the ground, (*d*) mature alfalfa plants, freshly harvested, (*e*) the green, growing portion of sphagnum plants, consisting largely of *Sph. acutifolium*. These plant materials were chopped into small pieces and sufficient quantities placed in large glazed earthenware pots to give 200 to 300 gm. of the dry plant substance for each pot. The material was then saturated with distilled water, an excess of free water always being present on the surface of the decomposing mixture. All the pots were then inoculated with a suspension of fresh garden soil, covered with plates, and incubated at 25 to 28°C.

At various intervals of time, the contents of each pot were removed and weighed, the liquid being measured separately; several aliquot portions of the material were then removed from both the solid and liquid portions for the various analyses. Some of the determinations; namely, ammonia, total nitrogen, total dry matter, and water-soluble substances, were made on the fresh portions of material; but the complete analyses were carried out with samples which had previously dried enough to remove the excess of water.

The methods of analyses were those previously described (9), with certain slight modifications. The results were calculated on the basis of the percentage of the residual material as well as on the total original material, allowance being made for the samples removed at different times. In other words, the results are reported in two ways: 1. On the percentage basis of the material left after various periods of decomposition; this enables one to determine the relative changes in the concentration of the various chemical constituents with the progress of decomposition; it also tends to show the progressive formation of the so-called "humus" material, or substances left and those newly formed with the advance of decomposition of various plants. 2. On the basis of the total concentration of the various chemical complexes in the original and in the decomposed material; these data help to visualize even more rapidly the nature of the transformation of the plant material, considered from the point of view of the various chemical complexes that go into its make-up. In dealing with a relatively small quantity of material, the removal of samples at the various periods will involve certain errors in the calculation of the total and relative composition of the residue, unless careful record is kept of the amounts removed at the various periods.

ANAEROBIC DECOMPOSITION OF CORN STALKS, RYE STRAW, AND OAK LEAVES

The corn stalks used in these experiments were rich in water-soluble substances, including reducing sugars and nitrogen compounds. The organic matter as a whole has undergone very rapid decomposition even under anaerobic conditions, but the rate and extent of decomposition are much less than

under aerobic conditions. A comparison of the results presented in tables 1 and 2 with those reported previously (6) on the aerobic decomposition of plant substances, shows that after 27 days, 20.19 per cent of the total material was decomposed under anaerobic conditions, whereas 36.46 per cent disappeared in that same period of time in the aerobic experiments. After 498 days decomposition, 38.18 per cent of the total organic matter was left in the anaerobic

TABLE 1

Chemical composition of corn stalks and decomposed residues, at different stages of decomposition, under anaerobic conditions

On the basis of dry material

CHEMICAL CONSTITUENTS	ORIGINAL CORN	MATERIAL AFTER DAYS OF INCUBATION		
		27	135	498
	per cent	*per cent*	*per cent*	*per cent*
Ether-soluble	1.80	1.31	2.63	1.78
Water-soluble	14.18	7.89	7.21	4.71
Hemicelluloses	17.63	16.76	17.43	16.27
Cellulose	29.67	28.51	20.40	26.03
Lignin	11.28	14.65	15.96	19.89
Crude protein	1.98	3.93	9.63	8.94
(Total N)	(0.73)	(0.94)	(1.79)	(1.70)

TABLE 2

Total decomposition of the various chemical constituents of corn stalks, under anaerobic conditions

ORGANIC CONSTITUENTS	ORIGINAL MATERIAL	MATERIAL LEFT AFTER DAYS OF DECOMPOSITION					
		27		135		498	
		Total residue	Per cent of original	Total residue	Per cent of original	Total residue	Per cent of original
	gm.	*gm.*		*gm.*		*gm.*	
Total dry material	203.00	162.00	79.81	132.50	65.27	77.50	38.18
Ether-soluble fraction	3.65	2.13	58.36	3.48	95.57	1.37	37.67
Water-soluble fraction	28.71	12.78	44.52	9.54	33.21	3.65	12.70
Hemicelluloses	35.79	27.13	75.79	23.09	64.50	12.61	35.22
Cellulose	60.24	46.14	76.60	28.53	47.35	20.17	33.48
Lignin	22.90	23.73	103.60	21.15	92.34	15.42	67.32
Crude protein	4.06	6.38	157.02	12.77	314.41	6.93	170.56

system, and only 22.30 per cent (residual organic matter calculated on the percentage basis of the original fresh plant substance) was left in the aerobic mixture after a shorter period of time, namely after 405 days. In other words, the aerobic decomposition of the same plant substances resulted in a considerably smaller residue than in the anaerobic transformation, as far as the disappearance of the organic material as a whole is concerned.

A comparison of the disappearance of the individual chemical constituents under both sets of conditions, brings out even more striking differences. Of

the three most important groups of chemical complexes in the corn stalks and leaves; namely, those of the hemicelluloses, celluloses, and lignins, the following corresponding quantities were decomposed within 27 days under aerobic conditions: 41.07, 43.56, and 0; whereas under anaerobic conditions, the following corresponding amounts have disappeared in the same period of time: 24.21, 23.40, and 0. In other words, within the first 4 weeks of decomposition, the complex polysaccharides decomposed twice as rapidly when air was freely admitted to the compost. The lignins were resistant to attack by microorganisms under both sets of conditions; however, when decomposition finally set in, it was much slower under the anaerobic conditions than under the

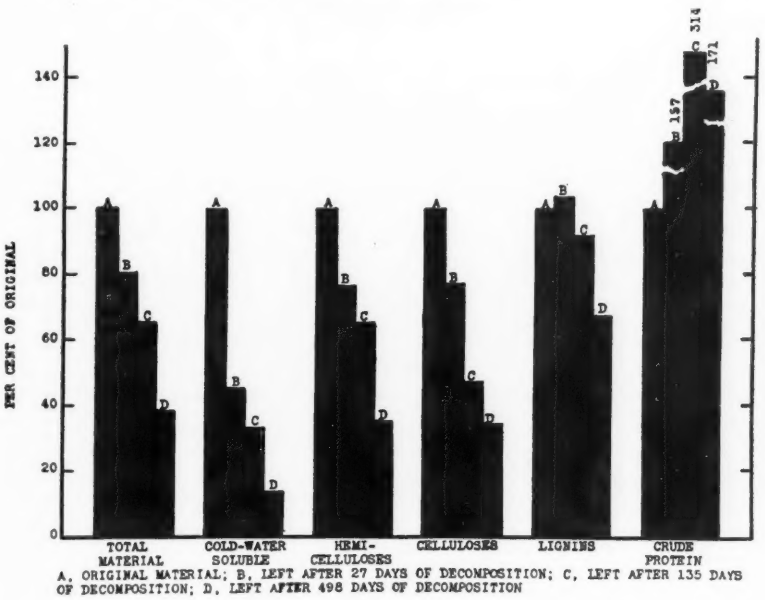

FIG. 1. ANAEROBIC DECOMPOSITION OF CORN STALKS

aerobic: 20.57 per cent of this group of complexes disappeared after 68 days in the aerobic system, but only 7.66 per cent of the lignins were lost after 135 days incubation under the anaerobic conditions; after 405 days, 42.93 per cent of the total original lignin material (including also the variously modified lignin complexes and their derivatives) was left in the aerobic system, whereas 67.32 per cent of this group of substances remained in the anaerobic system, even after 498 days decomposition. In other words, less than a third of the lignins disappeared after a period of nearly a year and a half, under the most favorable temperature conditions. One should mention in this connection that a heavy growth of fungi, actinomyces, and aerobic bacteria on the surfaces of the liquid

covering the plant material in the anaerobic system does not exclude the possibility that a certain amount of the plant constituents has undergone also an aerobic decomposition.

Figure 1 illustrates the gradual changes that have taken place in the process of transformation of corn stalks when saturated with water, thus excluding a rapid supply of air and favoring the development of anaerobic bacteria, in preference to fungi, actinomyces, and facultative aerobic bacteria which could

TABLE 3

Chemical composition of rye straw and decomposed residues, at different stages of decomposition, under anaerobic conditions

On basis of dry material

CHEMICAL CONSTITUENTS	ORIGINAL STRAW	MATERIAL AFTER DAYS OF INCUBATION		
		84	163	491
	per cent	*per cent*	*per cent*	*per cent*
Ether-soluble	1.84	1.97	1.59	1.47
Water-soluble	6.26	3.94	3.40	1.77
Hemicelluloses	21.10	20.36	19.48	19.30
Cellulose	38.52	35.05	31.31	34.81
Lignin	14.63	14.38	16.32	19.28
Crude protein	0.81	1.38	3.10	3.82
(Total N)	(0.28)	.....	(0.56)	(0.61)

TABLE 4

Total decomposition of the various chemical constituents of rye straw, under anaerobic conditions

ORGANIC CONSTITUENTS	ORIGINAL MATERIAL	MATERIAL LEFT AFTER DAYS OF DECOMPOSITION					
		84		163		491	
		Total residue	Per cent of original	Total residue	Per cent of original	Total residue	per cent of original
	gm.	*gm.*		*gm.*		*gm.*	
Total	276.90	257.20	92.89	217.30	78.48	181.24	65.43
Ether-soluble	5.10	5.05	99.02	3.53	69.12	2.71	53.14
Water-soluble	17.34	10.13	58.42	7.43	42.82	3.26	18.80
Hemicelluloses	58.42	52.33	89.57	42.19	72.21	34.90	59.74
Cellulose	106.66	90.14	84.51	68.03	63.78	62.92	58.99
Lignin	40.51	36.99	91.30	35.50	87.63	34.92	86.20
Crude protein	2.25	3.59	159.56	6.56	291.56	7.05	313.11

develop only on the surface of the liquid. The water-soluble substances, celluloses, and hemicelluloses have decomposed more rapidly than the total organic matter, whereas the lignins have decomposed much more slowly; the proteins or complex nitrogenous organic substances have accumulated not only relatively but also in actual concentrations when compared with the initial concentration of these complexes in the fresh plant material, taken as 100 per cent.

Results on the rapidity of aerobic and anaerobic decomposition of rye straw, similar to those obtained on the decomposition of corn stalks and leaves, are given in tables 3 and 4. Both the total and the individual chemical constituents of this plant material, with the exception of the cold-water-soluble substances, were found to decompose generally much more slowly than those of the corn stalks. Under aerobic conditions, 17 per cent of the straw decomposed in 66 days and 29 per cent in 143 days, whereas, under anaerobic conditions, 7 and 21.5 per cent were decomposed in 84 and 163 days respectively. The three most important chemical groups; namely, the celluloses, hemicelluloses, and lignins, were found to have decomposed also much more slowly under

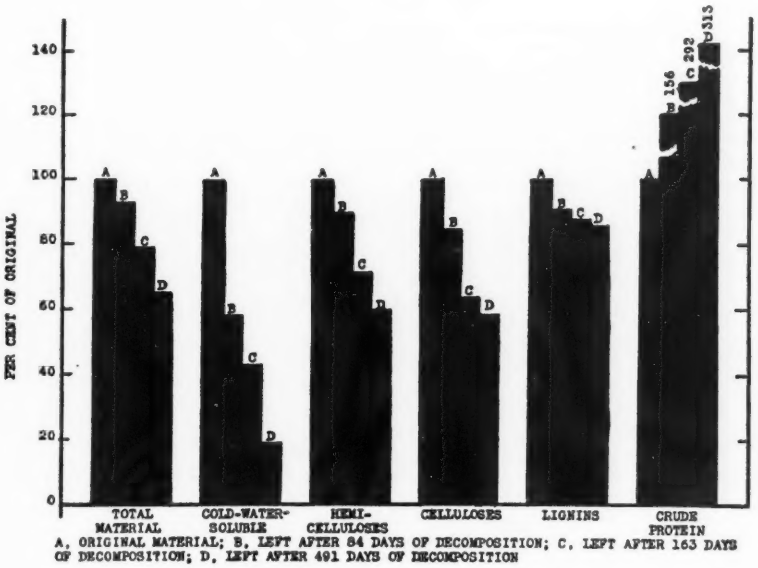

FIG. 2. ANAEROBIC DECOMPOSITION OF RYE STRAW

anaerobic than under aerobic conditions. In the aerobic system, in the absence of nutrient salts, 46.8 per cent of the hemicelluloses, 51.3 per cent of the celluloses, and 20.3 per cent of the lignins disappeared in a period of 386 days; the corresponding quantities of the chemical constituents of this plant substance that have decomposed under anaerobic conditions were 40.3, 41.0, and 13.8 per cent. Figure 2 illustrates graphically the changes that have taken place in the anaerobic decomposition of rye straw. The water-soluble substances were rapidly attacked, these were followed by the celluloses and hemicelluloses. The lignins were very resistant, and the organic nitrogenous compounds rapidly accumulated.

The difference in the rapidity and nature of decomposition of natural plant substances under the two sets of conditions is especially marked in the oak leaves (tables 5 and 6). More than twice as much of the total organic matter decomposed under aerobic conditions as under anaerobic. When the material was properly aerated (about 200 per cent moisture), 42.15 per cent of the total organic matter, 45.09 per cent of the hemicelluloses, 62.56 cellulose, and 15.03

TABLE 5

Chemical composition of oak leaves and decomposed residues, at different stages of decomposition, under anaerobic conditions

On the basis of dry material

CHEMICAL CONSTITUENTS	ORIGINAL LEAVES	MATERIAL AFTER DAYS OF DECOMPOSITION		
		84	163	491
	per cent	*per cent*	*per cent*	*per cent*
Ether-soluble	3.71	2.91	3.33	2.53
Water-soluble	13.93	5.83	4.49	2.12
Hemicelluloses	12.93	14.20	13.37	14.54
Cellulose	13.78	13.49	11.75	13.87
Lignin	30.30	33.42	35.74	40.32
Crude protein	4.25	5.94	6.05	7.29
(Total N)	(0.82)	(1.00)	(1.03)	(1.17)

TABLE 6

Total decomposition of the various chemical constituents of oak leaves, under anaerobic conditions

ORGANIC CONSTITUENTS	ORIGINAL MATERIAL	MATERIAL LEFT AFTER DAYS OF DECOMPOSITION					
		84		163		491	
		Total residue	Per cent of original	Total residue	Per cent of original	Total residue	Per cent of original
	gm.	*gm.*		*gm.*		*gm.*	
Total	222.60	204.39	91.82	199.50	89.70	180.10	80.75
Ether-soluble	8.27	5.98	72.31	6.65	80.35	4.56	55.08
Water-soluble	31.06	11.89	38.27	8.94	28.78	3.82	12.30
Hemicelluloses	28.85	29.00	100.52	27.66	92.41	26.19	90.77
Celluloses	30.73	27.58	89.74	23.43	76.25	24.97	81.26
Lignins	67.57	68.27	101.04	71.29	105.50	72.62	107.47
Crude proteins	4.25	12.17	286.36	12.07	284.00	13.12	308.71

lignin disappeared in 286 days. Under anaerobic conditions, the corresponding quantities that disappeared in 491 days were 19.25, 9.23, 18.76, and 0 per cent The lignins in the oak leaves did not decompose at all under anaerobic conditions; the hemicelluloses came next in slowness of decomposition.

The accumulation of proteins was in all cases greater under anaerobic than under aerobic conditions. This may be due to the more economic use of the nitrogen, to the smaller loss of ammonia, or to a smaller decomposition of the

synthesized proteins in the anaerobic system. Figure 3 shows the relatively marked preservation of the oak leaves, as a whole, under anaerobic conditions. The most marked phenomenon is the complete lack of decomposition of the lignins. Whether this is because the lignins of the oak leaves are of a different nature from those of corn stalks, since they have a higher fat and wax content, or because of some other factors, still remains to be determined. The much greater resistance of the hemicelluloses than of the celluloses in the case of this plant material also deserves special consideration. This is probably because the chemical composition of the hemicelluloses in oak leaves is quite markedly

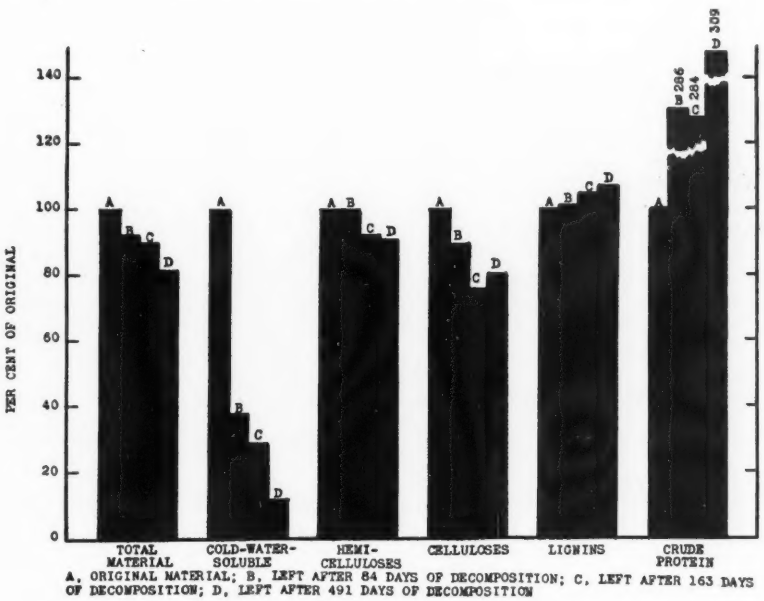

FIG. 3. ANAEROBIC DECOMPOSITION OF OAK LEAVES

different in the tree product from those in the corn residue or the cereal straw, as will be shown in a later publication.

DECOMPOSITION OF ALFALFA PLANTS

The alfalfa plant differed in chemical composition from the other plant materials, especially in its considerably higher nitrogen content. This material also underwent much less decomposition under anaerobic conditions than under aerobic. This is true not only of the total plant substance but also of the celluloses and hemicelluloses. The lignins of the alfalfa plant did not decompose at all in the anaerobic system within a period of 498 days, whereas in the aerobic

system, a marked decomposition of the lignin complexes took place, 40 per cent disappearing within 405 days (tables 7 and 8).

The proteins in the alfalfa underwent an immediate and rapid decomposition under the aerobic conditions; since the amount of nitrogen was greater than that required for synthetic activities by the microörganisms that bring about the decomposition of the celluloses and the hemicelluloses, a large part of the

TABLE 7

Chemical composition of alfalfa and decomposed residues, at different stages of decomposition, under anaerobic conditions

On the basis of dry material

CHEMICAL CONSTITUENTS	ORIGINAL ALFALFA	MATERIAL AFTER DAYS OF INCUBATION		
		27	135	498
	per cent	*per cent*	*per cent*	*per cent*
Ether-soluble	2.75	2.37	3.29	2.17
Water-soluble	17.24	12.18	13.63	6.74
Hemicelluloses	8.52	7.69	7.87	8.65
Cellulose	26.71	18.55	17.96	25.03
Lignin	10.78	14.89	18.61	23.67
Crude protein	8.13	14.91	21.44	8.51
(Total N)	(2.62)	(3.75)	(4.05)	(1.89)

TABLE 8

Total decomposition of the various chemical constituents of alfalfa, under anaerobic conditions

ORGANIC CONSTITUENTS	ORIGINAL MATERIAL	MATERIAL LEFT AFTER DAYS OF DECOMPOSITION					
		27		135		498	
		Total residue	Per cent of original	Total residue	Per cent of original	Total residue	Per cent of original
	gm.	*gm.*		*gm.*		*gm.*	
Total dry material	260.00	190.50	73.27	148.50	57.12	118.00	45.38
Ether-soluble fraction	7.15	4.52	63.15	4.90	68.54	2.56	35.73
Water-soluble fraction	44.82	23.19	51.74	20.24	45.16	7.95	17.74
Hemicelluloses	22.16	14.66	66.13	11.68	52.72	10.20	46.03
Cellulose	69.45	35.34	50.89	26.68	38.41	29.54	42.72
Lignin	28.03	28.37	101.21	27.64	98.59	27.93	99.64
Crude protein	21.14	28.40	134.32	31.86	150.71	10.04	47.47

nitrogen was liberated as ammonia and lost into the atmosphere. Under the anaerobic conditions, however, the nitrogen was largely preserved and only after 135 days of decomposition were any losses observed. This is brought out in table 9. The percentage of nitrogen in the compost at first increases, the total amount of nitrogen remaining constant. There was, however, a drop in the percentage of nitrogen as well as in the total nitrogen in the compost after

135 days of incubation. A rapid decrease in the amount of ammonia also began about that time. Figure 4 shows graphically the transformation of the alfalfa plant constituents when allowed to decompose in a medium saturated with water. The decomposition of the water-soluble constituents, celluloses,

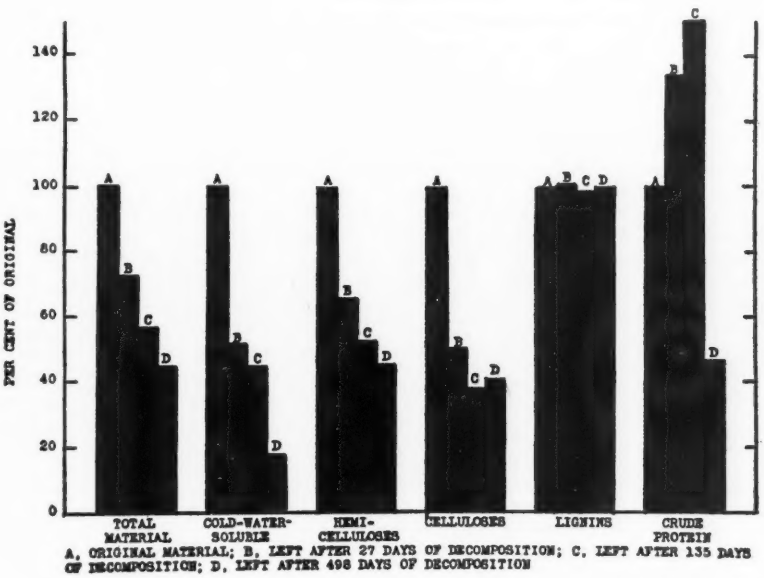

FIG. 4. TRANSFORMATION OF ALFALFA PLANT CONSTITUENTS IN A WATER-SATURATED MEDIUM

TABLE 9

Nitrogen losses in the decomposition of alfalfa under anaerobic conditions

PERIOD OF INCUBATION	N IN COMPOST	AMMONIA N IN COMPOST	RATIO OF NH_3-N TO TOTAL N	TOTAL N IN COMPOST
days	*per cent*	*per cent*	*per cent*	*gm.*
0	2.62	0.04	1.5	6.81
27	3.75	1.07	28.5	7.15
135	4.05	1.09	26.9	6.02
498	1.89	0.22	11.6	2.23

and hemicelluloses is similar in a way to the other plant substances; the complete preservation of the lignins is similar to the processes in the oak leaves, but the transformation of the organic nitrogenous compounds is quite characteristic of this plant substance.

AEROBIC AND ANAEROBIC DECOMPOSITION OF SPHAGNUM PLANTS

The decomposition of the sphagnum plants took place in a manner strikingly different from that of the other plant substances (tables 10 and 11). The decomposition of this material as a whole as well as of some of its chemical constituents was considerably slower than that of any other plant material previously tested; this was especially true under the anaerobic conditions, when less than 10 per cent of the total material disappeared within a period of 508 days. The most interesting phenomenon in the decomposition of the sphagnum plants is the fact that, next to the water-soluble substances, the proteins

TABLE 10

Chemical composition of sphagnum at different stages of decomposition under aerobic and anaerobic conditions

On the basis of dry material

CHEMICAL CONSTITUENTS	ORIGINAL SPHAGNUM	MATERIAL LEFT AFTER DECOMPOSITION			
		Aerobic		Anaerobic	
		163 days	508 days	163 days	508 days
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
Ether-soluble	1.11	1.23	1.07	1.46	1.50
Water-soluble (cold and hot)	6.95	1.95	2.39	1.56	0.97
Hemicelluloses	27.73	28.40	25.73	28.30	24.70
Cellulose	19.21	15.64	16.51	14.58	16.11
Lignin	7.33	7.43	8.73	8.26	9.86
Crude protein	5.50	4.81	4.41	5.35	3.85
Total N	1.10	1.05	0.86	1.06	0.71
NH_3-N	0.12	0.36	0.23	0.28	0.06

TABLE 11

Total decomposition of the various chemical constituents of sphagnum, under aerobic and anaerobic conditions

ORGANIC CONSTITUENTS	ORIGINAL MATERIAL	MATERIAL LEFT AFTER DECOMPOSITION							
		Aerobic				Anaerobic			
		163 days		508 days		163 days		508 days	
	gm.	*gm.*	*per cent*	*gm.*	*per cent*	*gm.*	*per cent*	*gm.*	*per cent*
Total	106.80	94.55	88.53	76.60	71.72	97.90	91.67	96.60	90.45
Ether-soluble	1.19	1.16	97.48	0.82	68.91	1.43	120.17	1.44	121.01
Water-soluble (cold and hot)	7.42	1.88	25.34	1.83	24.59	1.53	20.55	0.94	12.61
Hemicelluloses	29.62	26.82	90.53	19.72	66.58	27.70	93.52	23.85	80.52
Cellulose	20.54	14.78	72.00	12.64	61.58	14.27	69.55	15.56	75.83
Lignin	7.83	7.01	89.53	6.70	85.57	8.08	103.70	9.52	121.58
Crude protein	5.87	4.55	77.43	3.40	57.84	5.23	89.10	3.71	63.21
Total N	1.17	0.99	84.61	0.66	56.41	1.04	88.89	0.69	58.97
NH_3-N	0.14	0.34	242.86	0.18	128.57	0.27	192.86	0.06	42.86

or the organic nitrogenous compounds decomposed more rapidly than any other group of chemical constituents, both under aerobic and anaerobic conditions. This is quite contrary to the observations recorded for the other plant materials, when the proteins gradually accumulated, frequently to a concentration several times greater than in the original material. Only in the case of alfalfa, with a very high initial nitrogen content, did the organic nitrogen content diminish after several weeks. In the corn stalks, the initial nitrogen content of 0.73 per cent increased to 1.79 per cent of the residual material, after 135 days under the

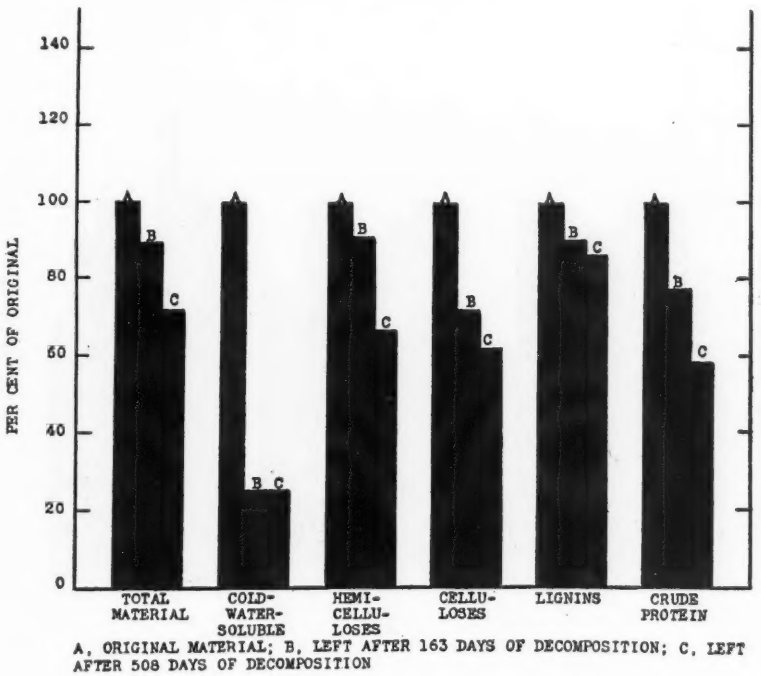

FIG. 5. AEROBIC DECOMPOSITION OF SPHAGNUM PLANTS

anaerobic conditions; in the oak leaves, with a rather limited total decomposition, the increase was from 0.82 to 1.17 per cent; in the sphagnum plants, however, under the same conditions, the nitrogen decreased from 1.17 per cent in the original undecomposed plants to 0.69 per cent in 508 days.

These marked differences in the behavior of the sphagnum plants explain two phenomena commonly observed in peat bogs; namely, the ability of the sphagnum plants to grow continuously in such a poor medium as the sphagnum bog, especially that of the highmoor type; and the formation of large quantities of ammonia as a result of decomposition of sphagnum peat (5, 9). In the

absence of plants that are capable of assimilating the ammonia formed as a result of the rapid decomposition of the nitrogenous compounds, the ammonia will at first accumulate and then gradually be lost into the atmosphere, since nitrifying bacteria are unable to live in such a highly acid medium as the sphagnum bog represents. This is clearly shown in the figures for total and relative quantities of total and ammonia nitrogen (table 11).

Next to the organic nitrogenous compounds in rapidity of decomposition of the various chemical constituents of the sphagnum plants came the celluloses;

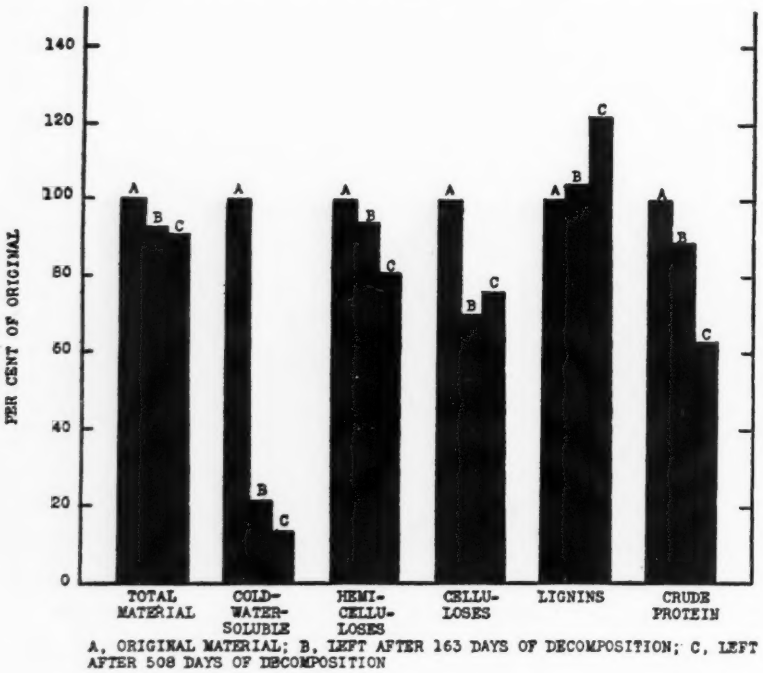

FIG. 6. ANAEROBIC DECOMPOSITION OF SPHAGNUM PLANTS

these were followed by the hemicelluloses. The ether-soluble substances and the lignins of the sphagnum plants were most resistant to decomposition. A certain amount of degradation of these two groups of complexes took place under aerobic conditions; in the anaerobic system, however, these two complexes did not decompose at all and actually increased not only relatively but absolutely. Whether this increase is due to an error in the experimental determination or whether substances of a similar nature were actually synthesized by the microörganisms in the anaerobic system still remains to be seen. Figures 5 and 6 illustrate the processes of transformation of the sphagnum plants

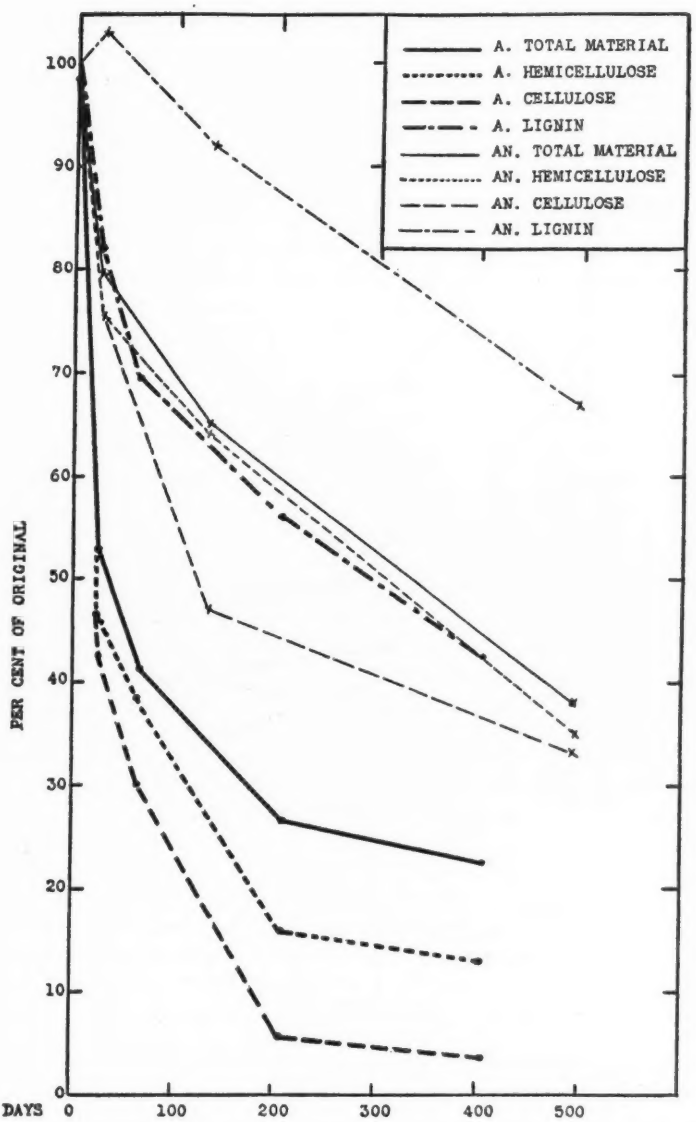

FIG. 7. COMPARISON OF ANAEROBIC (AN.) AND AEROBIC (A.) DECOMPOSITION OF CORN

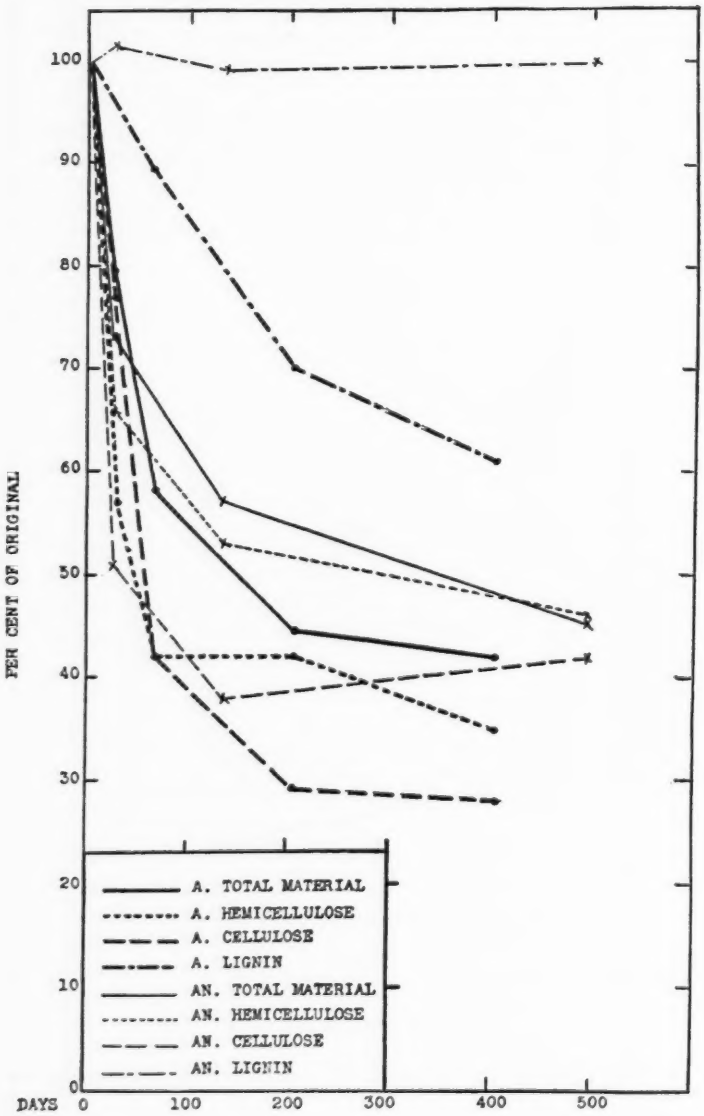

FIG. 8. COMPARISON OF ANAEROBIC (AN.) AND AEROBIC (A.) DECOMPOSITION OF ALFALFA

as a whole and of their various chemical constituents decomposing under aerobic and anaerobic conditions.

To permit a comparison at a glance of the difference in the rapidity of decomposition of two plant materials in a compost to which air was admitted freely (aerobic) and one saturated with water (anaerobic), the results for corn and alfalfa have been redrawn in figures 7 and 8. These figures help one to visualize further, also, the influence of the two sets of conditions upon the decomposition of the various chemical complexes in the two plant materials. Although the total amount of decomposition of the celluloses and hemicelluloses was more rapid than that of the entire material, the decomposition of the lignins was much slower, whereas the rate of accumulation of the organic nitrogenous compounds (proteins) was at first very rapid, then gradually diminished. The curve for protein accumulation is just the reverse of the curves of cellulose and hemicellulose decomposition. They just compensate one another, the former being directly dependent upon the other, the synthesis of the proteins being a direct result of the utilization of the polysaccharides as sources of energy by the microörganisms.

DISCUSSION

Under anaerobic conditions, plant materials as a whole decompose much more slowly than under aerobic conditions. The difference in the rapidity of decomposition of the various chemical constituents is even more striking. This is true especially of the lignins and organic nitrogenous complexes, when compared with the decomposition of the celluloses and hemicelluloses.

These results dealing with the decomposition of various plant materials under anaerobic conditions also explain fully the differences in the chemical composition of lowmoor and highmoor peats or between those peats that have been formed from the decomposition of herbaceous (*Cladium*, *Carex*, *Phragmites*) and of woody plants, on the one hand, and peat that has been formed largely from sphagnum plants, on the other hand. It will be recalled (8, 9) that the organic matter of lowmoor peats is characterized by a high protein and lignin content, and by a low content of celluloses (frequently none), hemicelluloses, and ether-soluble substances. On the other hand, the sphagnum peats are characterized by a low protein and a relatively low lignin content, and by high amounts of celluloses, hemicelluloses, and ether-soluble substances. The studies reported here on the anaerobic decomposition of corn stalks, rye straw, and oak leaves, on the one hand, and of sphagnum, on the other, readily explain these differences in the chemical composition of the two types of peat. The lowmoor peat is made up of plants in which the celluloses and hemicelluloses decompose rapidly while the lignins and proteins accumulate, the latter through the synthesizing agencies of microörganisms that use the polysaccharides as sources of energy. In the decomposition of sphagnum plants, low in lignins, in highmoors, the organic nitrogenous complexes are rapidly attacked; the nitrogen thereby made available cannot be utilized by microörganisms in

the absence of readily available sources of energy, since the polysaccharides of the sphagnum plants are rather resistant to decomposition.

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